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PTO/SB/05 (2/98) SEQ

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for nonprovisional applications under 37 CFR § 1.53(b))

Attorney Docket No.	860098.421C1
First Inventor or Application Identifier	Melanie Cobb
Title	TAO PROTEIN KINASE POLYPEPTIDES AND METHODS OF USE THEREFORE
Express Mail Label No.	EL487806495US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

1. [ ] General Authorization Form & Fee Transmittal *(Submit an original and a duplicate for fee processing)*

2. [X] Specification [Total Pages] 44 *(preferred arrangement set forth below)*
   - Descriptive Title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure

3. [X] Drawing(s) *(35 USC 113)* [Total Sheets] 20

4. Oath or Declaration [Total Pages]
   a. [ ] Newly executed (original or copy)
   b. [ ] Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional with Box 17 completed)*
      i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b)

5. [ ] Incorporation By Reference *(useable if box 4b is checked)* The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered to be part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. [ ] Microfiche Computer Program *(Appendix)*

7. Nucleotide and Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. [X] Computer-Readable Copy
   b. [X] Paper Copy (identical to computer copy)
   c. [X] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8. [ ] Assignment Papers (cover sheet & document(s))
9. [ ] 37 CFR 3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
10. [ ] English Translation Document *(if applicable)*
11. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
12. [ ] Preliminary Amendment
13. [X] Return Receipt Postcard
14. [ ] Small Entity Statement(s) [ ] Statement filed in prior application, Status still proper and desired
15. [ ] Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
16. [X] Other: Certificate of Express Mail

17. **If a CONTINUING APPLICATION,** *check appropriate box and supply the requisite information below and in a preliminary amendment*

[ ] Continuation [ ] Divisional [X] Continuation-In-Part (CIP) of prior Application No.: 09/060,410 filed 04/14/98

Prior application information: Examiner _____ Group / Art Unit _____

[ ] Claims the benefit of Provisional Application No. _____

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Melanie Cobb, Michele Hutchison, Zhu Chen and Kevin Berman
Filed	:	October 10, 2000
For	:	TAO PROTEIN KINASE POLYPEPTIDES AND METHODS OF USE THEREFORE

Docket No.	:	860098.421C1
Date	:	October 10, 2000

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Respectfully submitted,

STMicroelectronics, Inc.

Jeanette West

Enclosures:

Postcard
Diskette
Form PTO/SB/05
Specification Claims, Abstract (44 pages)
Declaration
Sequence Listing (29 pages)
20 Sheets of Drawings (Figures 1-20)

TAO PROTEIN KINASE POLYPEPTIDES AND METHODS OF USE THEREFOR

STATEMENT OF GOVERNMENT INTEREST

The Government owns certain rights in the present invention pursuant to NIH Grant GM53032.

CROSS-REFERENCE TO PRIOR APPLICATION

This application is a continuation-in-part of United States Patent Application No. 09/060,410, filed April 14, 1998.

TECHNICAL FIELD

The present invention relates generally to compositions and methods for modulating the activity of the MAP/ERK kinase MEK3 and/or other MEK family members. The invention is more particularly related to polypeptide variants of TAO proteins that have an enhanced ability to stimulate phosphorylation and activation of MEK substrates, such as MEK3. The invention is further related to the use of such proteins, for example, to activate a stress-responsive MAP kinase pathway in an organism and to identify antibodies and other agents that inhibit or activate signal transduction via such a pathway.

BACKGROUND OF THE INVENTION

MAP kinase pathways are conserved signal transduction pathways that activate transcription factors, translation factors and other target molecules in response to a variety of extracellular signals. Each pathway contains a MAP kinase module, consisting of a MAP kinase or ERK, a MAP/ERK kinase (MEK), and a MEK kinase (MEKK). In higher eukaryotes, activation of MAP kinase pathways has been correlated with cellular events such as proliferation, oncogenesis, development and differentiation. Accordingly, the ability to regulate signal transduction via these pathways could lead to the development of treatments and preventive therapies for human diseases associated with MAP kinase pathways, such as inflammatory diseases, autoimmune diseases and cancer.

Several MAP kinase pathways have been found in *S. cerevisiae* (Hunter and Plowman, *Trends in Biochem. Sci. 22*:18-22, 1997), and parallel mammalian pathways have

been identified based upon sequences of mammalian ERKs and yeast MAP kinases, KSS1 and FUS3 (Boulton et al., *Science* 249:64-67, 1990; Courchesne et al., *Cell 58*: 1107-1119, 1989; Elion et al., *Cell 60*:649-664, 1990). The best delineated yeast MAP kinase pathway, activated by mating pheromones, is controlled by a receptor-G protein system, includes a Cdc42 small G protein, and requires at least three protein kinases, Ste20p (Leberer et al., *EMBO J. 11*:4815-4828, 1992; Ramer et al., *Proc. Natl. Acad. Sci. USA 90*:452-456, 1993), Ste11p (Rhodes et al., *Genes Dev. 4*:1862-1874, 1990), and Ste7p (Teague et al., *Proc. Natl. Acad. Sci. USA 83*:7371-7375, 1986), upstream of the MAP kinase Fus3p (Elion et al., *Cell 60*:649-664, 1990).

Ste20p was isolated from *S. cerevisiae* as a gene whose product functions downstream of the βγ subunits of a heterotrimeric G protein but upstream of enzymes in the MAP kinase module (MEKK, MEK, ERK) of the pheromone response pathway (Leberer et al., *EMBO J. 11*:4815-4828, 1992; Ramer et al., *Proc. Natl. Acad. Sci. USA 90*:452-456, 1993). Ste11p, the MEKK, may be one of the Ste20p substrates (Wu et al., *J. Biol. Chem. 270*:15984-15992, 1990); thus, Ste20p-like enzymes may activate MEKKs in mammalian MAP kinase pathways. Ste20p, like its best studied mammalian counterparts, the p21-activated protein kinases (PAKs), is thought to be regulated by binding to Cdc42 through a conserved Cdc42/Rac interactive binding region, or CRIB domain (Burbelo et al., *J. Biol. Chem. 270*:29071-29074, 1995).

Mammalian relatives of Ste20p are diverse and include the PAK subfamily (PAK1,2,3) and the mixed lineage kinase (MLK) subfamily, including the dual leucine zipper kinase (DLK), germinal center kinase (GCK), and the Nck-interacting kinase, NIK. In the past year, newly identified Ste20p-related kinases include members of the MLK subfamily, SOK-1, Krs-1 and -2, and MUK. MUK was isolated in a screen for MEKK isoforms, but in fact shows more identity to MLK. In transfected cells several of these enzymes, as first shown with GCK, increase the activity of the stress-responsive kinases, particularly SAPK/JNK. In the case of NIK and GCK, they may work by binding to MEKK (Su et al., *EMBO J.* 16:1279-1290, 1997). However, several of these Ste20p-related enzymes also have MEKK activity. For example, DLK phosphorylates and potently activates MEKs that lie in the stress-responsive cascades.

Further characterization of members of these pathways, and the identification of additional members, is critical for understanding the signal transduction pathways involved and for developing methods for activating or inactivating MEKs and MAP kinase pathways *in vivo*. Accordingly, there is a need in the art for improved methods for modulating the activity of members of MAP kinase pathways, and for treating diseases associated with such pathways. The present invention fulfills these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides compositions and methods for modulating the activity of MAP/ERK kinases such as MEK3, and stress-responsive MAP kinase pathways. Within certain aspects, the present invention provides polypeptide variants of TAO proteins. Within one such aspect, polypeptide variants of TAO1 are provided, comprising an amino acid sequence that is at least 80% identical to residues 15-285 of SEQ ID NO:2, with the proviso that the variant does not comprise more than 500 consecutive amino acids of SEQ ID NO:2. Certain such variants comprise an amino acid sequence that is at least 90% identical to residues 15-285 of SEQ ID NO:2. Within certain embodiments, the variant comprises residues 1-416, 1-320 and/or 15-285 of SEQ ID NO:2.

Within other aspects, the present invention provides polypeptide variants of TAO2, comprising an amino acid sequence that is at least 80% identical to residues 15-285 of SEQ ID NO:4, with the proviso that the variant does not comprise more than 500 consecutive amino acids of SEQ ID NO:4. Certain such variants comprise an amino acid sequence that is at least 90% identical to residues 15-285 of SEQ ID NO:4. Within certain embodiments, the variant comprises residues 1-416, 1-320 and/or 15-285 of SEQ ID NO:4.

The present invention further provides, within other aspects, polypeptide variants of ceTAO, comprising an amino acid sequence that is at least 80% identical to residues 47-323 of SEQ ID NO:28, with the proviso that the variant does not comprise more than 500 consecutive amino acids of SEQ ID NO:28. Certain such variants comprise an amino acid sequence that is at least 90% identical to residues 47-323 of SEQ ID NO:28. Within certain embodiments, the variant comprises residues 1-454, 1-358 and/or 47-323 of SEQ ID NO:28.

Within further aspects, the present invention provides isolated polynucleotides encoding a polypeptide variant as described above. Certain such polynucleotides encode comprise at least 800 consecutive nucleotides any one of SEQ ID NOs:1, 3 or 27. Recombinant expression vectors comprising such a polynucleotide, as well as host cells transformed or transfected with such expression vectors are further provided.

Pharmaceutical compositions are also provided, within other aspects, comprising: (a) a polypeptide variant or polynucleotide as described above; and (b) a physiologically acceptable carrier.

The present invention further provides methods for phosphorylating a MEK polypeptide, comprising contacting a MEK polypeptide with a polypeptide variant as described above, wherein the MEK polypeptide comprises MEK3, MEK4 or MEK6 or a variant thereof, and thereby phosphorylating the MEK polypeptide.

Within further aspects, methods are provided for activating a member of a stress-responsive MAP kinase pathway in an organism, comprising administering to an organism a polypeptide variant as described above, and thereby activating a member of a stress-responsive MAP kinase pathway.

Within further aspects, methods are provided for screening for an agent that modulates signal transduction via a stress-responsive MAP kinase pathway, comprising: (a) contacting a candidate agent with a variant as described above; and (b) subsequently measuring the ability of the variant to modulate the activity of a MEK3 polypeptide, and thereby evaluating the ability of the compound to modulate signal transduction via a stress-responsive MAP kinase pathway.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents the nucleotide and predicted amino acid sequence of a representative TAO1 kinase (SEQ ID NOs: 1 and 2).

Figure 2 presents a comparison of the catalytic domains of TAO1 (residues 1-273 of SEQ ID NO:2), TAO2 (residues 1-273 of SEQ ID NO:4), STE20 (SEQ ID NO:17) and the *C. elegans* homolog (ceTAO) (SEQ ID NO:18). The catalytic domains were aligned by eye and the conserved amino acids bolded. The domains are indicated with roman numerals.

Figures 3A and 3B are Northern blots, showing TAO1 (Figure 3A) and TAO2 (Figure 3B) expression is various tissues. Various rat poly-A+ RNAs were probed, as indicated. Equal loading of RNA was verified by hybridizing the blot to an actin probe (not shown).

Figures 4A and 4B are Northern blots in which RNAs made from various human brain and spinal cord sections were hybridized to a TAO1-specific probe. Shown below each blot is the result of its hybridization to an actin probe. The lanes are as follows: 1, amygdala, 2, caudate nucleus, 3, corpus callosum, 4, hippocampus, 5, whole brain, 6, substantia nigra, 7, subthalamic nucleus, 8, thalamus, 9, cerebellum, 10, cerebral cortex, 11, medulla, 12, spinal cord, 13, occipital lobe, 14, frontal lobe, 15, temporal lobe, 16, putamen.

Figures 5A-5C are immunoblots. In Figure 5A, human embryonic kidney 293 cells were transiently transfected with either vector or pCMV5TAO1$(HA)_3$, and 24 hours later lysates were immunoblotted with a monoclonal antibody directed against the HA epitope. TAO1 is indicated by the arrow. In Figure 5B, the TAO1 proteins purified from Sf9 cells were immunoblotted with an antibody directed against the MRGS$(H)_6$ epitope. In Figure 5C, 50ng of $(His)_6$TAO1 was immunoblotted with polyclonal antisera P820 directed against a TAO1 peptide. An equal amount was blotted with the preimmune serum for P820.

Figure 6 is an autoradiogram showing the results of a representative *in vitro* linked kinase assay to estimate MEK activation by TAO1. Either 50 ng (lanes 1 and 3) or 250 ng (lanes 2 and 4) of $(His)_6$TAO1(1-416) was incubated with 50 ng of $(His)_6$MEK3 for one hour at 30° in the presence of Mg/ATP, after which a portion of the each reaction was added to a second reaction containing $(His)_6$p38. After a one hour incubation, the reactions were subjected to SDS-PAGE and autoradiography.

Figure 7 is an autoradiogram showing the results of a representative *in vitro* linked kinase assay to estimate MEK activation by TAO1. Only the second part of the linked assay is shown. The assay was identical to that described in Figure 6, except that GSTMEK4

was substituted for MEK3, and both $(His)_6$p38 and GSTSAPKβ were used as MEK4 substrates.

Figure 8 is an autoradiogram showing the results of a representative *in vitro* linked kinase assay to estimate MEK activation by TAO1. The assay was as described in Figures 6 and 7, but was performed with GSTMEK6 and $(His)_6$p38 as the MEK6 substrate.

Figure 9 is a histogram comparing the fold activations of MEKs 1 through 6 by $(His)_6$TAO1(1-416).

Figure 10 is an autoradiogram illustrating TAO1 activation of MEK3 *in vivo*. Human embryonic kidney 293 cells were transiently transfected with either vector alone, or pCMV5TAO1$(HA)_3$ and pCMV5mycMEK3, alone and in combination. Immunoprecipitates made with a monoclonal antibody directed against the myc epitope were subjected to *in vitro* kinase assays with $(His)_6$p38 as substrate. Myc-tagged MEK3 expression detected with a polyclonal anti-MEK3 antisera is shown below. In several separate experiments, MEK3 activity in the immunoprecipitates was increased 3 to 4 fold when coexpressed with TAO1.

Figure 11 is an autoradiogram illustrating the copurification of TAO1 and endogenous MEK3 from Sf9 cells. Either 100μg of Sf9 whole cell lysate, or 1μg each of the recombinant TAO1 proteins purified from Sf9 cells was Western blotted with polyclonal antisera directed against MEK3 (top panel) or MEK4 (lower panel). An identical Western blot performed with an antisera against MEK6 did not detect MEK6 protein in either the Sf9 lysate or the TAO1 preparations.

Figure 12 presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:5) with nts. 2341-2754 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 13 presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:6) with nts. 964-651 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 14 presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:7) with nts. 2792-2423 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 15A presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:8) with nts. 2248-2437 of the rat TAO1 kinase sequence (query) provided in Figure 1

(SEQ ID NO:1). Figure 15B presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:9) with nts. 2437-2501 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 16 presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:10) with nts. 2087-2305 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 17A presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:11) with nts. 3228-3312 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1). Figure 17B presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:12) with nts. 3200-3245 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 18 presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:13) with nts. 739-854 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 19A presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:14) with nts. 526-643 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1). Figure 19B presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:15) with nts. 187-296 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 20 presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:16) with nts. 866-733 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compounds and methods for modulating (*i.e.,* stimulating or inhibiting) the activity of MAP/ERK family members such as the MAP/ERK kinase MEK3. Compounds that activate such MEKs generally stimulate MEK phosphorylation. Such compounds include Ste20p homologs referred to herein as TAO proteins (*i.e.*. TAO1 (SEQ ID NO:2), TAO2 (SEQ ID NO:4), ceTAO (SEQ ID NO:28), as well as polypeptide variants of such proteins that retain the ability to stimulate MEK3 phosphorylation at a level that is not substantially lower than the

level stimulated by the native protein). Alternatively, a compound that activates MEK3 may comprise a polynucleotide that encodes a TAO polypeptide. Within other embodiments, compositions that stimulate MEK3 phosphorylation (thereby activating MEK3) may also, or alternatively, include one or more agents that stimulate TAO polypeptide expression or kinase activity. Such agents include, but are not limited to, stress-inducing agents (*e.g.*, DNA-damaging agents). Additional such agents may be identified by combining a test compound with a TAO polypeptide *in vitro* and evaluating the effect of the test compound on the kinase activity of the polypeptide using, for example, a representative assay described herein.

Preferred TAO polypeptides are those that comprise a C-terminal portion and have an activity (*i.e.*, the ability to stimulate MEK3 phosphorylation) that is comparable to, or enhanced relative to, a native TAO protein. Such polypeptides generally comprise at least the majority of the catalytic domain of a TAO protein (or a variant that is at least 80% identical the TAO protein catalytic domain), but do not comprise more than 500 consecutive amino acids of a TAO protein. For TAO1 or TAO2, a preferred polypeptide variant comprises residues 15-285; For ceTAO a preferred polypeptide variant comprises residues 47-323. It has been found, within the context of the present invention, that such truncated polypeptides may have substantially higher activity than the native TAO protein (*i.e.*, at least two fold higher, preferably at least 10 fold higher).

Compositions that inhibit the activity of MEKs generally inhibit MEK phosphorylation. Such compositions may include one or more agents that inhibit or block TAO polypeptide activity, such as an antibody that inhibits the kinase activity of a TAO polypeptide, a competing peptide that represents the substrate binding domain of a TAO protein or a phosphorylation motif of the MEK3 substrate, an antisense polynucleotide or ribozyme that interferes with transcription and/or translation of a TAO polypeptide, a molecule that inactivates a TAO polypeptide by binding to the polypeptide, a molecule that binds to the TAO substrate and prevents phosphorylation by a TAO polypeptide or a molecule that prevents transfer of phosphoryl groups from the kinase to the substrate. Agents that inhibit TAO polypeptide kinase activity may be identified by combining a test compound with a TAO polypeptide *in vitro* and evaluating the activity of the TAO polypeptide using a TAO kinase assay.

TAO POLYNUCLEOTIDES

Any polynucleotide that encodes a TAO polypeptide, or a portion or variant thereof as described herein, is encompassed by the present invention. Such polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a TAO polynucleotide may, but need not, be linked to other molecules and/or support materials. Preferred polynucleotides are those that encode a polypeptide having enhanced activity, relative to a native TAO protein.

Native TAO DNA sequences, or portions thereof, may be isolated using any of a variety of hybridization or amplification techniques, which are well known to those of ordinary skill in the art. Within such techniques, probes or primers may be designed based on the TAO sequences provided herein, and may be purchased or synthesized. Libraries from any suitable tissue (*e.g.,* brain) may be screened. An amplified portion or partial cDNA molecule may then be used to isolate a full length gene from a genomic DNA library or from a cDNA library, using well known techniques. Alternatively, a full length gene can be constructed from multiple PCR fragments.

Nucleic acid sequences corresponding to the native rat TAO polypeptides TAO1 and TAO2 are provided in SEQ ID NO:1 and SEQ ID NO:3, respectively; and the encoded amino acid sequences are provided in SEQ ID NOs:2 and 4, respectively. The predicted TAO1 open reading frame encodes a polypeptide of 1001 amino acids with a calculated molecular mass of 134 kD. TAO1 comprises an amino-terminal catalytic domain and an extensive carboxy-terminal region that has several distinguishing features, such as a possible nucleotide binding site and acidic stretch just carboxy-terminal to the catalytic domain, as well as two serine-rich regions. The TAO1 catalytic domain extends 263 amino acids from amino acid 25 to 288 with all 11 of the typical protein kinase subdomains conserved. There are two glutamate residues between TAO1 subdomains II and IV; the second glutamate at amino acid 76 contained in the sequence KEVK is most likely to represent subdomain III (Hanks et al., *Science 241*:42-52, 1988). The features of the TAO1 catalytic domain are most similar to the serine/threonine family of protein kinases;

subdomain VIb with the sequence HRDIKAGN (SEQ ID NO:26) suggests that TAO1 is likely to be a serine/threonine protein kinase. TAO2 has a similar arrangement of an amino-terminal kinase domain and a long carboxy-terminus, but differs in that it contains an acidic insert of 17 glutamate residues carboxy-terminal to the catalytic domain, and lacks the putative nucleotide binding site of TAO1.

As noted above, preferred polypeptide variants of TAO1 and TAO2 comprise an amino acid sequence that is at least 80% identical, and more preferably at least 90% identical, to residues 15-285 of a native TAO protein. Certain polypeptide variants comprise amino acids 1-320 or 1-416 of TAO1 or TAO2. Preferred polynucleotides encode such truncated variants, preferably variants with enhanced activity. For example, such TAO polynucleotides may comprise at least 800 consecutive nucleotides of a native sequence encoding TAO1 or TAO2.

ceTAO is the *Caenorhabditis elegans* TAO protein (Accession Number U32275; SEQ ID NO:28). Preferred variants of ceTAO comprise an amino acid sequence that is at least 80% identical, and more preferably at least 90% identical, to residues 47-323 of a native ceTAO. Certain such variants comprise amino acids 1-358 or 1-454 of ceTAO. Preferred polynucleotides encode such truncated variants, and particularly preferred TAO polynucleotides comprise at least 800 consecutive nucleotides of a native sequence encoding TAO1 or TAO2. Particularly preferred polynucleotides encode variants with enhanced activity.

The polynucleotides specifically recited herein, as well as full length polynucleotides comprising such sequences, other portions of full length polynucleotides, and sequences complementary to all or a portion of such full length molecules, are specifically encompassed by the present invention. In addition, TAO homologs from other species are specifically contemplated, and may generally be prepared as described herein for the rat homologs. In particular, within the context of the present invention, EST database sequences derived from retinal mRNAs have been identified that correspond to the human counterpart for TAO1. The sequences of these ESTs are provided in SEQ ID NOs:5-16. It will be readily apparent to those of ordinary skill in the art that a full length, native, human TAO1 polynucleotide may be identified based on such sequences, using for example, standard hybridization or amplification techniques. Such full length TAO1 sequences are

contemplated by the present invention, as are polypeptides encoded by such sequences, and variants of the naturally occurring sequences as discussed herein.

Polynucleotide variants of the recited sequences may differ from a native TAO polynucleotide in one or more substitutions, deletions, insertions and/or modifications. Certain variants encode a polypeptide that retains the ability to stimulate MEK3 phosphorylation at a level that is not substantially lower than the level stimulated by the native protein. The effect on the properties of the encoded polypeptide may generally be assessed as described herein. Preferred variants contain nucleotide substitutions, deletions, insertions and/or modifications at no more than 20%, preferably at no more than 10%, of the nucleotide positions. Certain variants are substantially homologous to a native gene, or a potion or complement thereof. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA sequence encoding a TAO protein (or a complementary sequence). Suitable moderately stringent conditions include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50° C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS). Such hybridizing DNA sequences are also within the scope of this invention.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention.

As noted above, the present invention further provides antisense polynucleotides and portions of any of the above sequences. Such polynucleotides may generally be prepared by any method known in the art, including synthesis by, for example, solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* or *in vivo* transcription of DNA sequences that are incorporated into a vector downstream of a suitable RNA polymerase promoter (such as T3, T7 or SP6). Certain portions of a TAO polynucleotide may be used to prepare an encoded polypeptide, as described herein. In addition, or alternatively, a portion may function as a probe (*e.g.,* to detect TAO expression in a sample), and may be labeled by a variety of reporter groups, such

as radionuclides, fluorescent dyes and enzymes. Such portions are preferably at least 10 nucleotides in length, and more preferably at least 20 nucleotides in length. Within certain preferred embodiments, a portion for use as a probe comprises a sequence that is unique to a TAO gene. A portion of a sequence complementary to a coding sequence (*i.e.,* an antisense polynucleotide) may also be used as a probe or to modulate gene expression. DNA constructs that can be transcribed into antisense RNA may also be introduced into cells or tissues to facilitate the production of antisense RNA.

Any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a vector will contain an origin of replication functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Additional initial, terminal and/or intervening DNA sequences that, for example, facilitate construction of readily expressed vectors may also be present. Suitable vectors may be obtained commercially or assembled from the sequences described by methods well-known in the art. Other elements that may be present in a vector will depend upon the desired use, and will be apparent to those of ordinary skill in the art.

Vectors as described herein may generally be transfected into a suitable host cell, such as a mammalian cell, by methods well-known in the art Such methods include calcium phosphate precipitation, electroporation and microinjection.

TAO POLYPEPTIDES

Polypeptides within the scope of the present invention comprise at least a portion of a TAO protein (*e.g.,* TAO1, TAO2 or ceTAO) or variant thereof, where the portion is immunologically and/or biologically active. Preferred variants retain the ability to stimulate MEK3 phosphorylation at a level that is not substantially lower than the level stimulated by the native protein. More preferably, a variant has enhanced ability to stimulate MEK3 phosphorylation (*e.g.,* at least two fold, five fold or ten fold), relative to the native protein. A polypeptide may further comprise additional sequences, which may or may not be derived from a native TAO protein. Such sequences may (but need not) possess immunogenic or antigenic properties and/or a biological activity.

A polypeptide "variant," as used herein, is a polypeptide that differs from a native protein in substitutions, insertions, deletions and/or amino acid modifications, such that the immunogenic and/or biological properties of the native protein are not substantially diminished. A variant preferably retains at least 80% sequence identity to a native sequence, more preferably at least 90% identity, and even more preferably at least 95% identity. Within certain preferred embodiments, such variants contain alterations at no more than 20% of the amino acid residues in the native polypeptide, such that the ability of the variant to stimulate MEK3 phosphorylation is enhanced. Guidance in determining which and how many amino acid residues may be substituted, inserted, deleted and/or modified without diminishing immunological and/or biological activity may be found using any of a variety of methods and computer programs known in the art. Properties of a variant may generally be evaluated by assaying the reactivity of the variant with, for example, antibodies as described herein and/or evaluating a biological property characteristic of the native protein.

A polypeptide is "immunologically active," within the context of the present invention if it is recognized (*i.e.,* specifically bound) by a B-cell and/or T-cell surface antigen receptor. Immunological activity may generally be assessed using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides derived from the native polypeptide for the ability to react with antigen-specific antisera and/or T-cell lines or clones, which may be prepared using well known techniques. An immunologically active portion of a TAO protein reacts with such antisera and/or T-cells at a level that is not substantially lower than the reactivity of the full length polypeptide (*e.g.,* in an ELISA and/or

T-cell reactivity assay). Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, 1988. B-cell and T-cell epitopes may also be predicted via computer analysis.

Similarly, a polypeptide is "biologically active" if the ability to phosphorylate MEK3 and/or other MEKs is not substantially diminished within a representative *in vitro* assay as described in Example 3. As used herein, the term "not substantially diminished" means retaining an activity that is at least 90% of the activity of a native TAO protein. Preferably, the ability of the polypeptide to phosphorylate MEK3 is enhanced at least two fold, preferably at least five fold and more preferably at least ten fold. Appropriate assays designed to evaluate such activity may be designed based on existing assays known in the art, and on the representative assays provided herein.

Preferred variants contain conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes.

In general, modifications may be more readily made in non-critical regions, which are regions of the native sequence that do not substantially change the properties of the TAO protein. Non-critical regions may be identified by modifying the TAO sequence in a particular region and assaying the activity of the resulting variant in a kinase assay, using MEK3, MEK4, MEK6 or another MEK family member as a substrate, as described herein.

Modifications may also be made in critical regions of a TAO protein, provided that the resulting variant retains the ability to stimulate MEK3 phosphorylation and/or an immunogenic property of the native protein. Inactive proteins may be created by modifying certain critical regions. One critical region comprises the aspartate 169 residue of TAO1 or TAO2. Modification of that residue results in a catalytically defective mutant. Another critical region encompasses the lysine 57 residue of TAO1 or TAO2. The effect of any modification on the ability of the variant to stimulate phosphorylation of MEK3 or other MEKs may generally be evaluated using any assay for TAO kinase activity, such as the representative assays described herein. Preferred variants with enhanced activity include those comprising an amino acid sequence that is at least 80% identical to residues 15-285, 1-320 or 1-426 of TAO1 or TAO; or residues 47-323, 1-358 or 1-454 of ceTAO/

Variants of TAO proteins may include constitutively active proteins. In general, activation of a TAO protein *in vivo* requires stimulation by a stimulus such as a stress-inducing agent. Constitutively active variants display the ability to stimulate MEK phosphorylation in the absence of such stimulation. Such variants may be identified using the representative *in vivo* assays for TAO kinase activity described herein.

TAO proteins may also be modified so as to render the protein constitutively inactive (*i.e.,* unable to phosphorylate MEKs even when stimulated as described above). Such modifications may be identified using the representative assays described herein. Genes encoding proteins modified so as to be constitutively active or inactive may generally be used in replacement therapy for treatment of a variety of disorders, as discussed in more detail below.

Variants within the scope of this invention also include polypeptides in which the primary amino acid structure of a native protein is modified by forming covalent or aggregative conjugates with other polypeptides or chemical moieties such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives may be prepared, for example, by linking particular functional groups to amino acid side chains or at the N- or C-termini.

The present invention also includes polypeptides with or without associated native-pattern glycosylation. Polypeptides expressed in yeast or mammalian expression systems may be similar to or slightly different in molecular weight and glycosylation pattern

than the native molecules, depending upon the expression system. Expression of DNA in bacteria such as *E. coli* provides non-glycosylated molecules. N-glycosylation sites of eukaryotic proteins are characterized by the amino acid triplet Asn-A_1-Z, where A_1 is any amino acid except Pro, and Z is Ser or Thr. Variants having inactivated N-glycosylation sites can be produced by techniques known to those of ordinary skill in the art, such as oligonucleotide synthesis and ligation or site-specific mutagenesis techniques, and are within the scope of this invention. Alternatively, N-linked glycosylation sites can be added to a polypeptide.

As noted above, polypeptides may further comprise sequences that are not related to an endogenous TAO protein. For example, an N-terminal signal (or leader) sequence may be present, which co-translationally or post-translationally directs transfer of the polypeptide from its site of synthesis to a site inside or outside of the cell membrane or wall (*e.g.,* the yeast α-factor leader). The polypeptide may also comprise a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.,* poly-His, hemaglutinin, glutathione-S-transferase or FLAG), or to enhance polypeptide stability or binding to a solid support. Protein fusions encompassed by this invention further include, for example, polypeptides conjugated to an immunoglobulin Fc region or a leucine zipper domain. All of the above protein fusions may be prepared by chemical linkage or as fusion proteins.

Also included within the polypeptides of the present invention are alleles of a TAO protein. Alleles are alternative forms of a native protein resulting from one or more genetic mutations (which may be amino acid deletions, additions and/or substitutions), resulting in an altered mRNA. Allelic proteins may differ in sequence, but overall structure and function are substantially similar.

TAO polypeptides, variants and portions thereof may generally be prepared from nucleic acid encoding the desired polypeptide using well known techniques. To prepare an endogenous protein, an isolated cDNA may be used. To prepare a variant polypeptide, standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis may be used, and sections of the DNA sequence may be removed to permit preparation of truncated polypeptides.

In general, any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides of this invention. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA sequence that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast, baculovirus-infected insect cells and animal cells. Following expression, supernatants from host/vector systems which secrete recombinant protein or polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. One or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

Portions and other variants having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc. 85*:2149-2146, 1963. Various modified solid phase techniques are also available (*e.g.*, the method of Roberge et al., *Science 269*:202-204, 1995). Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied BioSystems, Inc. (Foster City, CA), and may be operated according to the manufacturer's instructions.

In general, polypeptides and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, polypeptides provided herein are isolated to a purity of at least 80% by weight, more preferably to a purity of at least 95% by weight, and most preferably to a purity of at least 99% by weight. In general, such purification may be achieved using, for example, the standard techniques of ammonium sulfate fractionation, SDS-PAGE electrophoresis, and affinity chromatography. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

Antibodies and Fragments Thereof

The present invention further provides antibodies, and antigen-binding fragments thereof, that specifically bind to a TAO polypeptide. As used herein, an antibody, or antigen-binding fragment, is said to "specifically bind" to a TAO polypeptide if it reacts at a detectable level (within, for example, an ELISA) with a TAO polypeptide, and does not react detectably with unrelated proteins. Antibodies may be polyclonal or monoclonal. Preferred antibodies are those antibodies that inhibit or block TAO activity *in vivo* and within a kinase assay as described herein. Other preferred antibodies (which may be used, for example, in immunokinase assays) are those that immunoprecipitate active TAO1 and/or TAO2.

Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art (*see, e.g.,* Harlow and Lane, *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, 1988). In one such technique, an immunogen comprising the polypeptide is initially injected into a suitable animal (*e.g.,* mice, rats, rabbits, sheep and goats), preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for a TAO polypeptide may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol. 6*:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single

colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction.

Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by, for example, affinity chromatography on protein A bead columns.

<u>Methods and Kits for Detecting TAO Polypeptides and TAO Kinase Activity</u>

The present invention provides methods for detecting the level of TAO1 and/or TAO2 in a sample, as well as for detecting TAO kinase activity in a sample. The level of a TAO polypeptide or polynucleotide may generally be determined using a reagent that binds to the TAO protein, DNA or mRNA. To detect nucleic acid encoding a TAO protein, standard hybridization and/or PCR techniques may be employed using a nucleic acid probe or a PCR primer. Suitable probes and primers may be designed by those of ordinary skill in the art based on the TAO cDNA sequences provided herein. To detect TAO protein, the reagent is typically an antibody, which may be prepared as described herein.

There are a variety of assay formats known to those of ordinary skill in the art for using an antibody to detect a polypeptide in a sample. *See, e.g.,* Harlow and Lane, *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, 1988. For example, the antibody may be immobilized on a solid support such that it can bind to and remove the polypeptide from the sample. The bound polypeptide may then be detected using a second antibody that binds to the antibody/peptide complex and contains a detectable reporter group.

Alternatively, a competitive assay may be utilized, in which polypeptide that binds to the immobilized antibody is labeled with a reporter group and allowed to bind to the immobilized antibody after incubation of the antibody with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the antibody is indicative of the level of polypeptide within the sample. Suitable reporter groups for use in these methods include, but are not limited to, enzymes (*e.g.,* horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin.

For detecting an active TAO protein in a sample, an immunokinase assay may be employed. Briefly, polyclonal or monoclonal antibodies may be raised against a unique sequence of a TAO protein (such as amino acid residues 296-315, 403-418, 545-563 or 829-848) using standard techniques. A sample to be tested, such as a cellular extract, is incubated with the anti-TAO antibodies to immunoprecipitate a TAO protein, and the immunoprecipitated material is then incubated with a substrate (*e.g.,* MEK3) under suitable conditions for substrate phosphorylation. The level of substrate phosphorylation may generally be determined using any of a variety of assays, as described herein.

TAO kinase assays, for use in evaluating the polypeptide variants and other agents discussed herein, include any assays that evaluate a compound's ability to phosphorylate MEK3 or other MEKs, thereby rendering the MEK active (*i.e.,* capable of phosphorylating *in vivo* substrates such as p38). MEKs such as MEK3 for use in such methods may be endogenous proteins or variants thereof, may be purified or recombinant, and may be prepared using any of a variety of techniques that will be apparent to those of ordinary skill in the art. For example, cDNA encoding MEK3 may be cloned by PCR amplification from a suitable human cDNA library, using polymerase chain reaction (PCR) and methods well known to those of ordinary skill in the art. MEK3 may be cloned using primers based on the published sequence (Derijard et al., *Science 267*:682-685, 1995). MEK3 cDNA may then be cloned into a bacterial expression vector and the protein produced in bacteria, such as *E. coli*, using standard techniques. The bacterial expression vector may, but need not, include DNA encoding an epitope such as glutathione-S transferase protein (GST) such that the recombinant protein contains the epitope at the N- or C-terminus.

A TAO kinase assay may generally be performed as described herein. Briefly, a TAO polypeptide may be incubated with MEK3 and [γ-^{32}P]ATP in a suitable buffer (such as 50 mM HEPES pH 8, 10 mM $MgCl_2$, 1 mM DTT, 100 μM ATP) for 60 minutes at 30°C. In general, approximately 50 ng to 1 μg of the polypeptide and 50 ng recombinant MEK3, with 2-7 cpm/fmol [γ-^{32}P]ATP, is sufficient. Proteins may then be separated by SDS-PAGE on 10% gels and subjected to autoradiography. Incorporation of [^{32}P]phosphate into MEK3 may be quantitated using techniques well known to those of ordinary skill in the art, such as with a phosphorimager. To evaluate the substrate specificity of polypeptide variants, a kinase assay may generally be performed as described above except that other MEK substrates (*i.e.*, MEK1, 2, 4 or 6) are substituted for the MEK3.

To determine whether MEK3 phosphorylation results in activation, a coupled *in vitro* kinase assay may be performed using a substrate for MEK3, such as p38, with or without an epitope tag. p38 for use in such an assay may be prepared as described in Han et al., *J. Biol. Chem. 271*:2886-2891, 1996. Briefly, following phosphorylation of MEK3 as described above, the MEK3 (*e.g.*, 0.1-10 ng) may be incubated with p38 (*e.g.*, 10 μg/ml) and [γ-^{32}P]ATP in a kinase buffer as described herein. It should be noted that alternative buffers may be used and that buffer composition can vary without significantly altering kinase activity. Reactions may be separated by SDS-PAGE, visualized by autoradiography and quantitated using any of a variety of known techniques. Activated MEK3 will be capable of phosphorylating p38 at a level that is at least 5% above background using such an assay.

The present invention further provides kits for detecting TAO polypeptides and TAO kinase activity. Such kits may be designed for detecting the level of a TAO polypeptide or polynucleotide, or may detect phosphorylation of MEK3 in a direct kinase assay or a coupled kinase assay, in which the level of phosphorylation and/or the kinase activity of MEK3 may be determined. TAO polypeptides and TAO kinase activity may be detected in any of a variety of samples, such as eukaryotic cells, bacteria, viruses, extracts prepared from such organisms and fluids found within living organisms. In general, the kits of the present invention comprise one or more containers enclosing elements, such as reagents or buffers, to be used in the assay.

A kit for detecting the level of TAO polypeptide or polynucleotide typically contains a reagent that binds to TAO1 and/or TAO2 protein, DNA or RNA. To detect

nucleic acid encoding a TAO polypeptide, the reagent may be a nucleic acid probe or a PCR primer. To detect a TAO protein, the reagent is typically an antibody. The kit also contains a reporter group suitable for direct or indirect detection of the reagent (*i.e.*, the reporter group may be covalently bound to the reagent or may be bound to a second molecule, such as Protein A, Protein G, immunoglobulin or lectin, which is itself capable of binding to the reagent). Suitable reporter groups include, but are not limited to, enzymes (*e.g.*, horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. Such reporter groups may be used to directly or indirectly detect binding of the reagent to a sample component using standard methods known to those of ordinary skill in the art.

A kit for detecting TAO kinase activity based on measuring the phosphorylation of MEK3 generally comprises MEK3 in combination with a suitable buffer. A kit for detecting TAO kinase activity based on detecting MEK3 activity generally comprises MEK3 in combination with a suitable MEK3 substrate, such as p38. Optionally, the kit may additionally comprise a suitable buffer and/or material for purification of MEK3 after activation and before combination with substrate. Such kits may be employed in direct or coupled kinase assays, which may be performed as described above.

METHODS FOR IDENTIFYING BINDING AGENTS AND MODULATING AGENTS

The present invention further provides methods for identifying antibodies and other compounds that bind to and/or modulate the activity of a TAO polypeptide. To evaluate the effect of a candidate modulating agent on TAO polypeptide activity, a kinase assay may be performed as described above, except that the candidate modulating agent is added to the incubation mixture. Briefly, the reaction components, which include the composition to be tested and the TAO polypeptide or a polynucleotide encoding the kinase, are incubated under conditions sufficient to allow the components to interact. Subsequently, the effect of composition on kinase activity or on the level of a polynucleotide encoding the kinase is measured. The observed effect on the kinase may be either inhibitory or stimulatory. The increase or decrease in kinase activity can be measured by, for example, adding a radioactive compound such as ^{32}P-ATP to the mixture of components, and observing radioactive incorporation into MEK3 or other suitable substrate for a TAO

polypeptide, to determine whether the compound inhibits or stimulates kinase activity. A polynucleotide encoding the kinase may be inserted into an expression vector and the effect of a composition on transcription of TAO mRNA can be measured, for example, by Northern blot analysis.

Within such assays, the candidate agent may be preincubated with a TAO polypeptide before addition of ATP and substrate. Alternatively, the substrate may be preincubated with the candidate agent before the addition of kinase. Further variations include adding the candidate agent to a mixture of TAO polypeptide and ATP before the addition of substrate, or to a mixture of substrate and ATP before the addition of TAO polypeptide. Any of these assays can further be modified by removing the candidate agent after the initial preincubation step. In general, a suitable amount of antibody or other candidate agent for use in such an assay ranges from about 0.1 μM to about 10 μM. The effect of the agent on TAO kinase activity may then be evaluated by quantitating the incorporation of [^{32}P]phosphate into MEK3, as described above, and comparing the level of incorporation with that achieved using the TAO polypeptide without the addition of the candidate agent.

TAO kinase activity may also be measured in whole cells transfected with a reporter gene whose expression is dependent upon the activation of MEK3. For example, polynucleotides encoding a TAO polypeptide and a substrate (*e.g.,* MEK3) may be cotransfected into a cell. The substrate may then be immunoprecipitated, and its activity evaluated in an *in vitro* assay. Alternatively, cells may be transfected with a ATF2-dependent promoter linked to a reporter gene such as luciferase. In such a system, expression of the luciferase gene (which may be readily detected using methods well known to those of ordinary skill in the art) depends upon activation of ATF2 by p38, which may be achieved by the stimulation of MEK3 with a TAO polypeptide. Candidate modulating agents may be added to the system, as described below, to evaluate their effect on TAO polypeptide activity.

Alternatively, a whole cell system may employ only the transactivation domain of ATF2 fused to a suitable DNA binding domain, such as GHF-1 or GAL4. The reporter system may then comprise the GH-luciferase or GAL4-luciferase plasmid. Candidate TAO protein modulating agents may then be added to the system to evaluate their effect on ATF2-specific gene activation.

In other aspects of the subject invention, methods for using the above polypeptides to phosphorylate and activate MEK3, peptide derivatives thereof or other MEK family members are provided. MEK substrate for use in such methods may be prepared as described above. In one embodiment, MEK3 may be phosphorylated *in vitro* by incubation with a TAO polypeptide and ATP in a suitable buffer as described above. In general, the amounts of the reaction components may range from about 0.1 μg to about 10 μg of TAO polypeptide, from about 0.1 μg to about 10 μg of recombinant MEK3, and from about 100 nM to about 1 mM (preferably about 100 pmol - 30 nmol) of ATP. Phosphorylated proteins may then be purified by binding to GSH-Sepharose and washing. The extent of MEK3 phosphorylation may generally be monitored by adding [γ-^{32}P]ATP to a test aliquot, and evaluating the level of MEK3 phosphorylation as described above. The activity of the phosphorylated MEK3 may be evaluated using a coupled *in vitro* kinase assay, as described above.

Once activated *in vitro*, MEK3 may be used, for example, to identify agents that inhibit the kinase activity of MEK3. Such inhibitory agents, which may be antibodies or drugs, may be identified using the coupled assay described above. Briefly, a candidate agent may be included in the mixture of MEK3 and p38, with or without pre-incubation with one or more components of the mixture, as described above. In general, a suitable amount of antibody or other agent for use in such an assay ranges from about 0.1 μM to about 10 μM. The effect of the agent on MEK3 kinase activity may then be evaluated by quantitating the incorporation of [^{32}P]phosphate into p38, as described above, and comparing the level of incorporation with that achieved using activated MEK3 without the addition of a candidate agent.

Within other aspects, TAO polypeptides may be used to identify one or more native upstream kinases (*i.e.,* kinases that phosphorylate and activate TAO1 and/or TAO2 *in vivo*, or other signaling molecules that regulate TAO activity). TAO polypeptides may be used in a yeast two-hybrid system to identify interacting proteins. Alternatively, an expression library may be screened to identify cDNAs that encode proteins which phosphorylate a TAO polypeptide. Other methods for identifying such upstream kinases may also be employed, and will be apparent to those of ordinary skill in the art.

PHARMACEUTICAL COMPOSITIONS

For administration to a patient, one or more polypeptides, polynucleotides, antibodies and/or modulating agents are generally formulated as a pharmaceutical composition, which may be a sterile aqueous or non-aqueous solution, suspension or emulsion, and which additionally comprises a physiologically acceptable carrier (*i.e.,* a non-toxic material that does not interfere with the activity of the active ingredient). Any suitable carrier known to those of ordinary skill in the art may be employed in a pharmaceutical composition. Representative carriers include physiological saline solutions, gelatin, water, alcohols, natural or synthetic oils, saccharide solutions, glycols, injectable organic esters such as ethyl oleate or a combination of such materials. Such compositions may also comprise buffers (*e.g.,* neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.,* glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, antimicrobial compounds, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide), inert gases and/or preservatives. Compositions of the present invention may also be formulated as a lyophilizate. Pharmaceutical compositions may also contain other compounds, which may be biologically active or inactive.

The compositions described herein may be administered as part of a sustained release formulation (*i.e.,* a formulation such as a capsule that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide or modulating agent dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of release. The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Certain pharmaceutical compositions contain DNA encoding a polypeptide, antibody fragment or other modulating agent as described above (such that a TAO

polypeptide, a variant thereof or a modulating agent is generated *in situ*) or an antisense polynucleotide. In such pharmaceutical compositions, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid, bacterial and viral expression systems, as well as colloidal dispersion systems, including liposomes. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). The DNA may also be "naked," as described, for example, in Ulmer et al., *Science 259*:1745-1749, 1993.

Various viral vectors that can be used to introduce a nucleic acid sequence into the targeted patient's cells include, but are not limited to, vaccinia or other pox virus, herpes virus, retrovirus, or adenovirus. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus including, but not limited to, Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a gene that encodes the ligand for a receptor on a specific target cell (to render the vector target specific). For example, retroviral vectors can be made target specific by inserting a nucleotide sequence encoding a sugar, a glycolipid, or a protein. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art.

Viral vectors are typically non-pathogenic (defective), replication competent viruses, which require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids that encode all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR, but that are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsulation. Such helper cell lines include (but are not limited to) Ψ2, PA317 and PA12. A retroviral vector introduced into such cells can be packaged and vector virion produced. The vector virions produced by this method can then be used to infect a tissue cell line, such as NIH 3T3 cells, to produce large quantities of chimeric retroviral virions.

Another targeted delivery system for TAO polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.,* an artificial membrane vesicle). It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 μm can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., *Trends Biochem. Sci. 6*:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, et al., *Biotechniques 6*:882, 1988).

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticuloendothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

Routes and frequency of administration, as well as polypeptide, modulating agent or nucleic acid doses, will vary from patient to patient. In general, the pharmaceutical compositions may be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity or transdermally. Between 1 and 6 doses may be administered

daily. A suitable dose is an amount of polypeptide or DNA that is sufficient to show improvement in the symptoms of a patient afflicted with a disease associated with a stress-responsive MAP kinase pathway. Such improvement may be detected based on a determination of relevant cytokine levels (*e.g.*, IL-2, IL-8), by monitoring inflammatory responses (*e.g.*, edema, transplant rejection, hypersensitivity) or through an improvement in clinical symptoms associated with the disease. In general, the amount of polypeptide present in a dose, or produced *in situ* by DNA present in a dose, ranges from about 1 μg to about 250 μg per kg of host, typically from about 1 μg to about 60 μg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 10 mL to about 500 mL for 10-60 kg animal.

Therapeutic Applications

The above polypeptides, polynucleotides and/or modulating agents may be used to phosphorylate (and thereby activate) MEK3, or to inhibit such phosphorylation, in a patient. As used herein, a "patient" may be any mammal, including a human, and may be afflicted with a disease associated with a stress-responsive MAP kinase pathway, or may be free of detectable disease. Accordingly, the treatment may be of an existing disease or may be prophylactic. Diseases associated with a stress-responsive MAP kinase pathway include any disorder which is etiologically linked to a TAO protein kinase activity, including immune-related diseases (*e.g.*, inflammatory diseases, autoimmune diseases, malignant cytokine production or endotoxic shock), cell growth-related diseases (*e.g.*, cancer, metabolic diseases, abnormal cell growth and proliferation or cell cycle abnormalities) and cell regeneration-related diseases (*e.g.*, cancer, degenerative diseases, trauma, environmental stress by heat, UV or chemicals or abnormalities in development and differentiation). Immunological-related cell proliferative diseases such as osteoarthritis, ischemia, reperfusion injury, trauma, certain cancers and viral disorders, and autoimmune diseases such as rheumatoid arthritis, diabetes, multiple sclerosis, psoriasis, inflammatory bowel disease, and other acute phase responses may also be treated.

Treatment includes administration of a composition or compound which modulates the kinase activity of TAO1 and/or TAO2. Such modulation includes the suppression of TAO expression and/or activity when it is over-expressed, or augmentation of

TAO expression and/or activity when it is under-expressed. Modulation may also include the suppression of phosphorylation of MEK3 or related kinases.

As noted above, antibodies, polynucleotides and other agents having a desired effect on TAO expression and/or activity may be administered to a patient (either prophylactically or for treatment of an existing disease) to modulate the activation of MEK3 *in vivo*. For example, an agent that decreases TAO activity *in vivo* may be administered to prevent or treat inflammation, autoimmune diseases, cancer or degenerative diseases. In particular, such agents may be used to prevent or treat insulin-resistant diabetes, metabolic disorders and neurodegenerative diseases. In general, for administration to a patient, an antibody or other agent is formulated as a pharmaceutical composition as described above. A suitable dose of such an agent is an amount sufficient to show benefit in the patient based on the criteria noted above.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1

Cloning and Sequencing cDNA Encoding TAO1 and TAO2

This Example illustrates the cloning of cDNA molecules encoding the rat Ste20p-related protein kinases TAO1 and TAO2, and the identification of the human TAO1 homolog.

First-strand cDNA from adult rat brain was used as the template in the first round of PCR with degenerate oligonucleotide primers derived from the Ste20p sequence, 5'-GACGCTGGATCCAA(AG)AT(ACT)GGICA(AG)GGIGC-3' (SEQ ID NO:19) and 5'-GGIGTICC(AG)TTIGTIGCIAT-3' (SEQ ID NO:20). A portion of the product of this reaction was used as the template in a second round of PCR with nested primers, also derived from the Ste20p sequence, 5'-AA(AG)GA(AG)CAIATI(CA)TIAA(CT)GA(AG)AT-3' (SEQ ID NO:21) and 5'-GACGCTGAATTCAC(CT)TCIGGIGCCATCCA-3' (SEQ ID NO:22). The resulting 420 base product was labeled with [α^{-32}P]dCTP by random-priming, and used to probe approximately $1x10^6$ plaques of an oligo(dT) and random-primed λZAP library generated from adult rat forebrain RNA. In excess of 100 positive clones were obtained; of those sequenced, all contained regions of overlap with the original PCR product. A full length TAO1 sequence was assembled from two overlapping cDNAs, using the SacI site at nucleotide 50 to insert a fragment of TAO1 cDNA including nucleotides 50 to 3003. The full length TAO1 sequence is shown in Figure 1 and SEQ ID NO:1.

The TAO1 open reading frame encodes 1001 amino acids, with a calculated molecular mass of 134kDa. The presumed initiator codon begins at base 121 and is preceded by an in-frame stop codon at base 106. The longest 5' UTR obtained was 600 nucleotides in length, and the longest 3' UTR was 1200 nucleotides. None of the clones analyzed contained a poly-A track.

As is the case with most protein kinases, TAO1 can be divided into regions based on amino acid sequence comparison to other protein kinases. TAO1 is composed of an amino-terminal catalytic domain and an extensive carboxy-terminal region that has several distinguishing features, such as a possible nucleotide binding site and acidic stretch just

carboxy-terminal to the catalytic domain, as well as two serine-rich regions. TAO1 does not appear to contain the leucine zipper motifs found in the MLK subfamily of kinases.

The TAO1 catalytic domain extends 263 amino acids from amino acid 25 to 288 with all 11 of the typical protein kinase subdomains conserved. There are two glutamate residues between TAO1 subdomains II and IV; the second glutamate at amino acid 76 contained in the sequence KEVK is most likely to represent subdomain III (Hanks et al., *Science 241*:42-52, 1988). The features of the TAO1 catalytic domain are most similar to the serine/threonine family of protein kinases; subdomain VIb with the sequence HRDIKAGN suggests that TAO1 is likely to be a serine/threonine protein kinase.

When using FASTA (GCG, Wisconsin Package) to align TAO1 with sequences from the databases, the TAO1 catalytic domain shows the highest degree of identity to a *C. elegans* putative serine/threonine protein kinase (accession number U32275), to which it has 63% identity and 79% similarity. That sequence appears to represent the *C. elegans* homolog of TAO1, and is shown as ceTAO in Figure 2. The TAO1 catalytic domain is 39% identical to Ste20p and 40% identical to the catalytic domains of the p21-activated kinases PAK1 and PAK2. The catalytic domain of TAO1 is only 31% identical to the mixed lineage kinase MLK1, and 33% identical to dual leucine zipper-bearing kinase (DLK), also known as MLK2. Thus, TAO1 appears to be more closely related to the STE20-like kinases than to the MLK family. TAO1 is also related to germinal center kinase (GCK) and mammalian Ste20-like kinase 1 (MST1), with 42% and 45% identity respectively in the catalytic domains. The TAO1 sequence has similarity with that of the MEK kinase MEKK1. Although the overall identity between the catalytic domains of TAO1 and MEKK is only 33%, the identity of the carboxy-terminal half of their catalytic domains is higher (42%).

In the process of screening the cDNA library for clones near the 5' end of TAO1, multiple clones representing a second closely related gene (TAO2) were identified. The TAO2 sequence is provided in SEQ ID NO:3, with the predicted amino acid sequence shown in SEQ ID NO:4 and Figure 2. TAO2 is highly related to TAO1, and has a similar arrangement of an amino-terminal kinase domain and a long carboxy-terminus, but differs in that it contains an acidic insert of 17 glutamate residues carboxy-terminal to the catalytic domain, and lacks the putative nucleotide binding site of TAO1.

Sequences from EST databases derived from retinal mRNAs revealed the human counterpart for TAO1. The EST sequences identified are provided in SEQ ID NOs:5-16, and the alignments of these sequences with the rat TAO1 sequence are provided in Figures 12-20.

The FASTA program was used to compare the percent amino acid identities of several protein kinase catalytic domains, and the results are presented in Table 1, below.

Table 1

	TAO1	TAO2	ceTAO	STE20	GCK	MLK1	MST1
TAO2	90						
ceTAO	65	61					
STE20d	40	39	37				
GCK	43	42	35	40			
MLK1	32	30	27	30	29		
MST1	47	43	42	42	47	28	
MEKK1	34	33	27	30	30	30	29

To assess the expression of TAO1 in transfected cells, full-length, HA-tagged TAO1 cDNA was transfected into human embryonic kidney 293 cells. A protein of approximately 140kDa could be detected by Western blotting with an antibody directed against the HA epitope (Figure 5A). The observed molecular mass of the protein is in good agreement with the mass predicted from the cDNA sequence.

Example 2

In vivo Expression of TAO1 and TAO2

This Example illustrates the expression of TAO1 and TAO2 in a variety of adult rat and human tissues, as determined by Northern blot analysis.

Total RNA isolated from various adult male rat tissues was selected for poly-A+ RNA with oligo(dT)cellulose (Collaborative Biomedical Products) according to the manufacturers protocols, and 5μg of each RNA was subjected to Northern analysis. The PCR-generated 420 base fragment derived from the catalytic domain of TAO1 (described above) was labeled with [α^{-32}P]dCTP by random-priming and used to probe the Northern blot. Hybridization was at 42°C, followed by washing at 55°C in 0.2%SSC/0.1%SDS. Integrity of the mRNA was confirmed by hybridization to an actin probe. The TAO1 probe hybridized predominately to an mRNA species of approximately 12kb, and less strongly to another of approximately 10kb (Figure 3A). Of the rat tissues examined, brain clearly showed the strongest hybridization signal. On prolonged exposure, heart and lung revealed weak hybridization signals, while in skeletal muscle, liver, kidney, testis, epididymus, and spleen no signal was detected.

To assess the expression pattern of TAO2, the rat tissue Northern blot was stored until the hybridization signal for TAO1 was not seen on a two week exposure at -80°C. A fragment from the catalytic domain of TAO2 was labeled with [α^{-32}P]dCTP by random priming, and used to probe the Northern under the same hybridization and washing conditions described above for TAO1.

When the same rat tissue Northern blot was probed with a fragment of the catalytic domain of TAO2, the strongest hybridization signal was also seen in brain. The size of the transcript hybridizing to the TAO2 probe was smaller than that seen for TAO1, at 5kb (Figure 3B).

A probe from the non-catalytic carboxy-terminus of TAO1 (corresponding to nucleotides 1555 to 2632 of TAO1 (*see* Figure 1)) was used for all additional Northern analyses because it is less likely to hybridize to TAO2 mRNA. This probe from the carboxy-terminus of TAO1 was used to assess the expression pattern in sections of human brain (Clontech). Hybridizations were performed at 68°C in Clontech ExpressHyb buffer, and washed at 55°C as per the manufacturer's instructions.

The strongest hybridization signals were seen in amygdala, corpus callosum, hippocampus, and substantia nigra, and each of these was stronger than that seen in whole brain (Figure 4A). Weaker signals were seen in caudate nucleus, subthalamic nucleus and thalamus. A second human brain Northern hybridized to the same probe showed strong

hybridization signals in cerebellum, putamen and occipital, frontal and temporal lobes, but much weaker signals in cerebral cortex, medulla and spinal cord (Figure 4B).

Example 3
Kinase Activity and Substrate Specificity of TAO1

This Example illustrates the kinase activity and substrate specificity of TAO1, in *in vitro* and *in vivo* assays.

To determine whether TAO1 is active as a protein kinase, two constructs were employed. pCMV5TAO1-HA_3 and pCMV5TAO1(1-416)-HA_3 were generated by cloning the cDNAs encoding these TAO1 polypeptides into the pCMV5 mammalian expression vector. Oligonucleotide primers were used with TAO1 cDNA as template to amplify a 1247 base pair DNA product encoding amino acids 1 to 416. This fragment contains all 11 of the kinase subdomains (with the initial methionine deleted). The resulting constructs were transfected into human embryonic kidney 293 cells, and the recombinant, tagged proteins immunoprecipitated with an antibody directed against the HA epitope.

In vitro kinase assays were generally performed as follows. Kinase assays contained: 50mM Hepes, pH 8, 10mM $MgCl_2$, 1mM DTT, 100μM ATP, [γ-^{32}P]ATP (at a final concentration of 2-7 cpm/fmol), and unless otherwise noted, reactions were incubated at 30°C for 60 minutes in a 30μl volume. Protein kinase substrates such as myelin basic protein were added at a final concentration of 0.5 mg/ml. Reactions were halted by the addition of 10μl 5X Laemmli buffer, followed by boiling, and 20μl were analyzed by SDS-PAGE and autoradiography. For linked kinase assays, 50-250ng of recombinant TAO1 protein was incubated with 50ng of each of the bacterially expressed MEK proteins in a 30μl reaction volume for 60 minutes at 30°C, and then 5μl of this reaction was added to a second reaction mix containing bacterially expressed $(His)_6$p38 or GST-SAPKβ at a final concentration of 10μg/ml. Recombinant MEK proteins were kindly provided by Andrei Khokhlatchev and Megan Robinson, and may be prepared as described by Robinson et al., *J. Biol. Chem. 271*:29734-29739, 1996 and references cited therein. Within such assays, both TAO1(1-416) and full-length TAO1 were able to phosphorylate MBP in immune complex kinase reactions.

To quantitate the activity of more highly purified TAO1, TAO1(1-416), full-length TAO1 and full-length TAO1(D169A) were expressed with an amino-terminal hexa-histidine tag in Sf9 cells. TAO1(D169A) is a catalytically defective TAO1 mutant, which was created by changing aspartic acid 169 to an alanine (D169A) with PCR, and cloning the resulting construct into the pCMV5 mammalian expression vector. These constructs were prepared with either a single hemaglutinin (HA) epitope tag at the amino-terminus, a triple HA epitope tag at the carboxy-terminus, or a myc epitope tag at the amino-terminus.

The recombinant, hexa-histidine tagged TAO1, TAO1(1-416), and TAO1(D169A) were expressed in *Spodoptera frugiperda* (Sf9) cells. Cells were lysed by douncing in 50mM sodium phosphate, pH 8.5, 1mM DTT, 1mM PMSF, and 1mg/ml each leupeptin, pepstatin A, and aprotinin. After centrifugation at 30,000xg for 30 minutes, the supernatant was applied to a Ni^{2+}- NTA agarose (Qiagen) column pre-equilibrated with the same buffer. The column was then washed with 50 column volumes of buffer, and eluted with a 20 ml gradient of 0 to 250mM imidazole, all in the above buffer. Fractions containing recombinant TAO1 proteins were detected in fractions by Western blotting with an antibody to the MRGS$(H)_6$ epitope (Qiagen), and appropriate fractions were pooled and dialyzed to remove the imidazole.

$(His)_6$TAO1(1-416) expressed as a single 57kDa band (Figure 5B). Both the $(His)_6$TAO1 and $(His)_6$TAO1(D169A) recombinant proteins migrated as 140kDa bands, although the D169A mutant appears to be more subject to degradation. $(His)_6$TAO1(1-416) phosphorylates MBP with a specific activity of 1 $\mu molmin^{-1}mg^{-1}$ in the presence of 1mM ATP. Full-length $(His)_6$TAO1 exhibits MBP phosphorylating activity that is comparable to the 1-416 truncation mutant, while the activity of TAO1(D169A) is reduced to 90% of that of the wild-type protein. $(His)_6$TAO1(1-416) was also able to phosphorylate α-casein, histone 1, and histone 7.

To determine whether TAO1 activates one or more of the known MEKs, $(His)_6$TAO1(1-416) was incubated with bacterially produced MEK for one hour in the presence of Mg^{2+} and [γ^{-32}P]ATP. A portion of this reaction was then transferred to a similar reaction containing the appropriate bacterially expressed MEK substrate, $(His)_6$ERK2K52R for MEK1 and MEK2, $(His)_6$p38 for MEK3 and MEK6, and $(His)_6$p38 and GST-SAPKβ for

MEK4. After a one hour incubation, the phosphoproteins were separated by SDS-PAGE. Autoradiography revealed that $(His)_6$TAO1(1-416) phosphorylated and activated $(His)_6$MEK3, and enhanced the ability of MEK3 to phosphorylate p38 by approximately 100-fold (Figure 6).

$(His)_6$TAO1(1-416) activated GST-MEK4 5-fold toward $(His)_6$p38, and 150-fold towards GST-SAPKβ (Figure 7). The difference in fold activation seen for MEK4 towards the two substrates probably reflects the difference in basal kinase activity of MEK4 towards p38 and SAPKβ *in vitro*. TAO1 also increased the ability of GST-MEK6 to phosphorylate $(His)_6$p38, by 5-fold (Figure 8). Recombinant GST-MEK5 was not phosphorylated by $(His)_6$TAO1(1-416).

Recombinant $(His)_6$TAO1 and $(His)_6$TAO1(D169A) were also examined for their ability to activate the same MEK proteins. $(His)_6$TAO1 showed a reduced ability to activate MEK3 as compared to that of the carboxy-terminal truncation mutant $(His)_6$TAO1(1-416). In multiple experiments, the full-length TAO protein displayed from 0 to 30% of the MEK3 activating ability of $(His)_6$TAO1(1-416), and $(His)_6$TAO1(D169A) was unable to activate any of the MEK proteins above basal activities.

The degree of activation of each of the MEK proteins by $(His)_6$TAO1(1-416) *in vitro* is comparable to that seen by a bacterially produced amino-terminal truncation of MEKK1 (Xu et al., *Proc. Natl. Acad. Sci. USA 92*:6808-6812, 1995; Robinson et al., *J. Biol. Chem. 271*:29734-29739, 1996). To distinguish the MEK-activating ability of TAO1 from that of MEKK, the ability of $(His)_6$TAO1(1-416) to activate MEK1 and MEK2 was assessed. As shown in Figure 9, $(His)_6$TAO1(1-416) was completely unable to increase the activity of MEK1 or MEK2 towards the substrate $(His)_6$ERK2 under the same conditions that TAO1 activates MEK3, MEK4, and MEK6. Thus, while TAO1 displays MEKK-like activity in its ability to activate various MEKs, TAO1 is differentiated from MEKK by its inability to recognize MEK1 and MEK2. Figure 9 shows the fold activation of the various MEKs by TAO1.

To assess the ability of TAO1 to activate the various MEKs *in vivo*, full-length HA-tagged TAO1 was co-transfected into 293 cells with myc-tagged MEK3, or myc-tagged TAO1 was co-transfected with HA-tagged MEK4 or HA-tagged MEK6. The pCMV5myc-MEK3 construct was generated by inserting the MEK3 coding sequence

(provided by K.L. Guan, University of Michigan, which may be prepared as described by Robinson et al., *J. Biol. Chem. 271*:29734-29739, 1996) into the pCMV5Myc vector, such that the Myc epitope is at the amino-terminus of MEK3. The MEKs were then immunoprecipitated and added to immune complex kinase assays with the appropriate substrate and Mg^{2+}/ATP. In multiple experiments, myc-tagged MEK3 showed a 3-fold higher activity toward p38 when immunoprecipitated from 293 cells co-expressing TAO than from cells not transfected with TAO (Figure 10). In contrast, TAO was not able to increase the activity of immunoprecipitated HA-tagged MEK4 towards GST-SAPKβ, or that of HA-tagged MEK6 toward p38.

In transfected cells, TAO1 activates MEK3 3-fold, but neither MEK4 nor MEK6. The selectivity in transfected cells may arise from the ability of TAO1 to bind MEK3. The endogenous MEK3 from Sf9 cells copurifies with recombinant TAO1 expressed in the cells. These findings suggest that TAO1 may be an important regulator of the p38 pathway.

To determine which MEK3 residues are phosphorylated by TAO, an *in vitro* kinase reaction was performed with $(His)_6$TAO1(1-416) and $(His)_6$MEK3; the 57kDa band corresponding to TAO1 and the 30 kDa band corresponding to MEK3 were excised and treated as described. Phosphoproteins were separated by SDS-PAGE, transferred to Immobilon-P membrane (Millipore) electrophoretically, and visualized by autoradiography. Bands of interest were excised and hydrolyzed in 6M HC1 for 60 minutes at 110°C. The hydrolysate was dried under vacuum, and resuspended in a 2.2% formic acid, 12% acetic acid solution at an activity of 2000cpm/μl. Then 1μl of each sample was mixed with 1μg each of the three phosphoamino acid standards, and spotted onto cellulose thin-layer chromatography plates. Electrophoresis was performed in 0.5% pyridine, 5% acetic acid at 1200 volts for 60 minutes. After air drying the plates, the standards were visualized with 0.25% ninhydrin in acetone. Autoradiography revealed only phosphoserine and phosphothreonine in both $(His)_6$TAO1(1-416) and $(His)_6$MEK3 (Figure 11).

Example 4
Co-Purification of MEK3 and TAO1

This Example shows that TAO1 and MEK3 co-purify.

Although the ability of $(His)_6$TAO1 to activate MEK3 was always reduced in comparison with that of $(His)_6$TAO1(1-416), several assays showed that the ability of $(His)_6$TAO1 to lead to an increase in the phosphorylation of p38 in the linked kinase assays was partly independent of the addition of MEK. $(His)_6$TAO1(1-416) does not phosphorylate p38. Therefore, Western analyses were performed to determine if one or more MEKs might be present in the TAO1 preparations purified from Sf9 cells.

$(His)_6$TAO1, $(His)_6$TAO1(1-416), and $(His)_6$TAO1(D169A) were subjected to Western analysis with antisera specific to MEK3, MEK4, and MEK6. Four different polyclonal antisera were raised to these three TAO1 peptides in rabbits. The peptide TKDAVRELDNLQYRKMKKLL (SEQ ID NO:23) corresponding to the amino acids 296 to 315 yielded antisera P820. The peptide KKELNSFLESQKREYKLRK (SEQ ID NO:24) of amino acids 545 to 563 yielded the antiserum R562. Finally, the peptide RELRELEQRVSLRRALLEQK (SEQ ID NO:25) of amino acids 829 to 848 resulted in the antisera R564 and R565. These peptides were conjugated to *Limulus* hemocyanin (Boulton and Cobb, *Cell. Regul.* *2*:357-371, 1991) and dialyzed into phosphate-buffered saline. A total of five boosts were performed, after which the rabbits were exsanguinated and the serum collected. The antisera were screened for reactivity by Western blotting of recombinant TAO1 expressed in Sf9 cells. Five antisera were found to consistently recognize the recombinant TAO1 protein in Western blots. Free peptide was able to block the specific recognition of TAO1 protein by the antisera. None of the five antisera detected the presence of TAO1 in lysates of 293, NIH3T3, NG-108, or COS cells.

For immunoblot analysis, either 50ng of recombinant TAO1 protein or 100 μg of cell lysate was subjected to SDS-PAGE, then transferred to nitrocellulose membranes. The membranes were blocked with 5% nonfat powdered milk in TBST (20mM Tris, pH 8, 500mM NaC1, 0.05% Tween 20) for one hour, then incubated with the polyclonal antisera at 1:500 dilution in TBST plus 0.25% milk for one hour. After three washes with TBST, the membranes were incubated with a 1:2500 dilution of horseradish peroxidase-conjugated

goat-anti-rabbit IgG in TBST plus 0.25% milk for one hour. Membranes were washed again in TBST then visualized with the ECL system (Amersham).

MEK3 was clearly seen in the $(His)_6$TAO1 preparation, and to a lesser extent in the $(His)_6$TAO1(D169A) preparation (Figure 11). MEK4 was detected in the Sf9 cell lysates, but not in the TAO1 preparations, while MEK6 was detected in neither.

Example 5
TAO Polypeptide Variants with Enhanced Activity

This Example illustrates the characterization of certain constitutively active TAO protein variants.

PBluescript-TAO2(1-320), containing the catalytic domain of TAO2 was generated by PCR. Wild-type TAO2 and TAO2(1-320) were cloned into pTSETB (Invitrogen) to incorporate a $MRGSH_6$ tag and were subsequently transferred to the baculovirus shuttle vector pVL1393. Recombinant viruses were selected, and recombinant protein was harvested, as described by Hutchison et al., *J. Biol. Chem. 273*:28625-28632, 1998.

Proteins were adsorbed to Ni^{+2}-nitrilotriacetic acid agarose (Qiagen) and eluted with a gradient of 20-250 mM imidazole in 0.5 mM dithiothreitol (DTT) and 0.3 M NaCl. TAO2 was detected by Western blotting with an antibody to the $MRGSH_6$ epitope (Qiagen) and silver staining.

Activity was assessed using *in vitro* kinase assays as described above, using 0.5 mg/mL myelin basic protein (MBP) as the substrate. The truncated, recombinant TAO2 (1-320) phosphorylated MBP with a specific activity of 0.6 $\mu mol\ min^{-1}\ mg^{-1}$. The full length protein had lower activity, about 10% of the truncated enzyme.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

Claims

1. A polypeptide variant of TAO1, comprising an amino acid sequence that is at least 80% identical to residues 15-285 of SEQ ID NO:2, with the proviso that the variant does not comprise more than 500 consecutive amino acids of SEQ ID NO:2.

2. A variant according to claim 1, wherein the amino acid sequence is at least 90% identical to residues 15-285 of SEQ ID NO:2.

3. A variant according to claim 1, wherein the variant comprises residues 1-416 of SEQ ID NO:2.

4. A variant according to claim 1, wherein the variant comprises residues 1-320 of SEQ ID NO:2.

5. A variant according to claim 1, wherein the variant comprises residues 15-285 of SEQ ID NO:2.

6. A polypeptide variant of TAO2, comprising an amino acid sequence that is at least 80% identical to residues 15-285 of SEQ ID NO:4, with the proviso that the variant does not comprise more than 500 consecutive amino acids of SEQ ID NO:4.

7. A variant according to claim 6, wherein the amino acid sequence is at least 90% identical to residues 15-285 of SEQ ID NO:4.

8. A variant according to claim 6, wherein the variant comprises residues 1-416 of SEQ ID NO:4.

9. A variant according to claim 6, wherein the variant comprises residues 1-320 of SEQ ID NO:4.

10. A variant according to claim 6, wherein the variant comprises residues 15-285 of SEQ ID NO:4.

11. A polypeptide variant of ceTAO, comprising an amino acid sequence that is at least 80% identical to residues 47-323 of SEQ ID NO:28, with the proviso that the variant does not comprise more than 500 consecutive amino acids of SEQ ID NO:28.

12. A variant according to claim 11, wherein the amino acid sequence is at least 90% identical to residues 47-323 of SEQ ID NO:28.

13. A variant according to claim 11, wherein the variant comprises residues 1-454 of SEQ ID NO:28.

14. A variant according to claim 11, wherein the variant comprises residues 1-358 of SEQ ID NO:28.

15. A variant according to claim 11, wherein the variant comprises residues 47-323 of SEQ ID NO:28.

16. An isolated polynucleotide encoding a polypeptide according to any one of claims 1-15.

17. An isolated polynucleotide according to claim 16, wherein the polynucleotide comprises at least 800 consecutive nucleotides of SEQ ID NO:1.

18. An isolated polynucleotide according to claim 16, wherein the polynucleotide comprises at least 800 consecutive nucleotides of SEQ ID NO:3.

19. An isolated polynucleotide according to claim 16, wherein the polynucleotide comprises at least 800 consecutive nucleotides of SEQ ID NO:27.

20. A recombinant expression vector comprising a polynucleotide according to claim 16.

21. A host cell transformed or transfected with an expression vector according to claim 20.

22. A pharmaceutical composition, comprising:

(a) a variant according to any one of claims 1-15; and

(b) a physiologically acceptable carrier.

23. A pharmaceutical composition, comprising:

(a) a polynucleotide according to claim 16; and

(b) a physiologically acceptable carrier.

24. A method for phosphorylating a MEK polypeptide, comprising contacting a MEK polypeptide with a variant according to any one of claims 1, 6 or 10, wherein the MEK polypeptide comprises MEK3, MEK4 or MEK6 or a variant thereof, and thereby phosphorylating the MEK polypeptide.

25. A method for activating a member of a stress-responsive MAP kinase pathway in an organism, comprising administering to an organism a variant according to any one of claims 1, 6 or 10, and thereby activating a member of a stress-responsive MAP kinase pathway.

26. The method of claim 25 wherein the member of the stress-responsive MAP kinase pathway is MEK3.

27. A method for screening for an agent that modulates signal transduction via a stress-responsive MAP kinase pathway, comprising:

(a) contacting a candidate agent with a variant according to any one of claims 1, 6 or 10; and

(b) subsequently measuring the ability of the variant to modulate the activity of a MEK3 polypeptide, and thereby evaluating the ability of the compound to modulate signal transduction via a stress-responsive MAP kinase pathway.

TAO PROTEIN KINASE POLYPEPTIDES AND METHODS OF USE THEREFOR

Abstract of the Disclosure

Compositions and methods are provided for potentiating the activity of the mitogen-activated protein kinase p38. In particular the mitogen-activated protein kinase kinase MEK6, and variants thereof that stimulate phosphorylation of p38 are provided. Such compounds may be used, for example, for therapy of diseases associated with the p38 cascade and to identify antibodies and other agents that inhibit or activate signal transduction via p38.

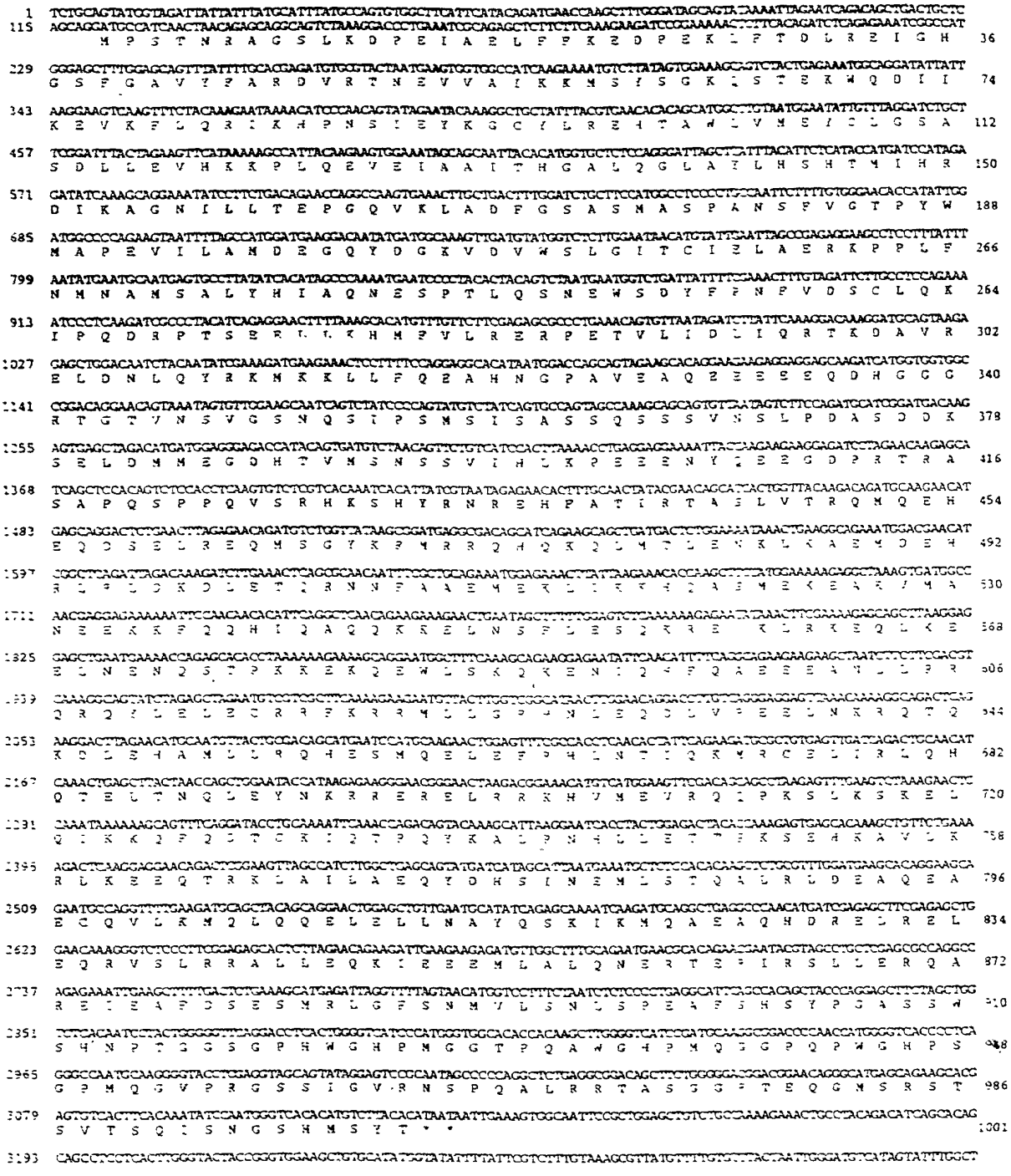

FIG. 1

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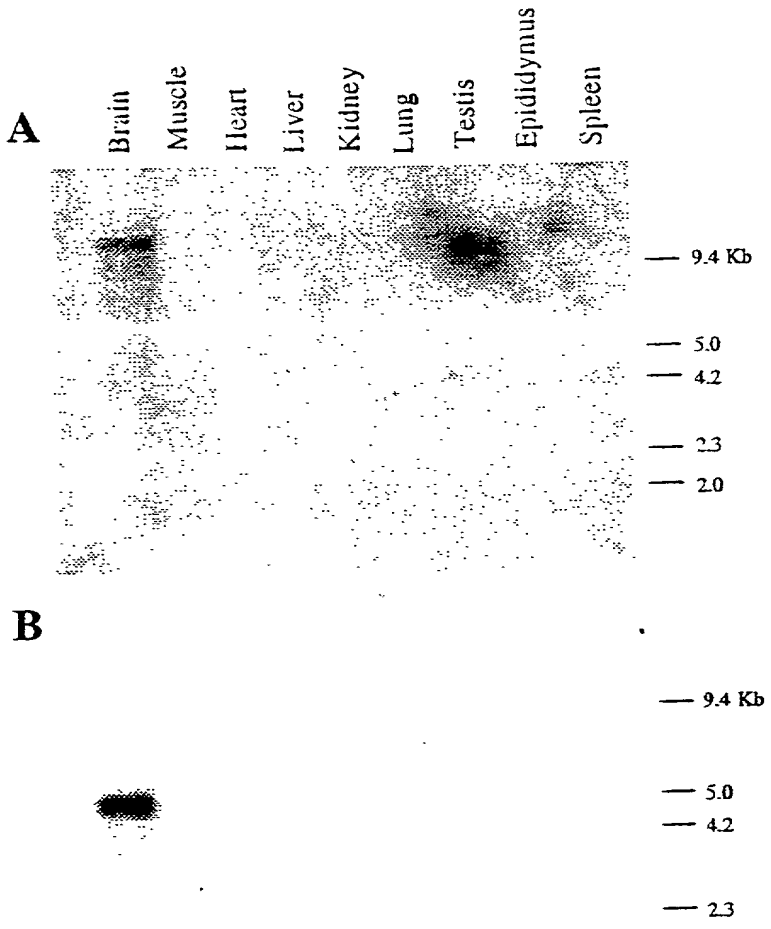
                                                I              II          III
TAO1   MPSTHRAGSLKDPEIAELFFKEDPEKLFTDLREIGHGSFGAVYFARDVRTNEVVAIKKMSYSGKQSTEKW--QDIIKEV  77
TAO2   MPAGGRAGSLKDPDVAELFFKDDPEKLFSDLREIGHGSFGAVYFARDVRNSEVVAIKKMSYSGKQSNEKW--QDIIKEV  77
ceTAO  -MAPAVLQKPGVIKDPSIAALFSNKDPEQDLREIGHGSFGAVYFAYDKKNEQTVAIKKMNFSGKQAVEKW- NDILKEV  76
STE20  KEERERRKKQLYAKLNEICSDGDPSTKYANLVKIGQGASGGVYTAYEIGTNVSVAIKQMNLE-KQPKKELIINEILVMK 670

                 IV                     V                                   VI
TAO1   KFLQRIKHPNSIEYKGCYLREHTAWLVMEYCLGSASDLLEVHKKPLQEVEIAAITHGALQGLAYLHSHTMIHRDIKAGN 156
TAO2   RFLQKLRHPNTIQYRGCYLREHTAWLVMEYCLGSASEFLEVHKKPLQEVEIAAVTHGALQGLAYLHSHNMIHRDVKAGN 156
ceTAO  SFLNTVVHPHIVDYKACFLKDTTCWLVMEYCIGSAADIVDVLRKGMREVEIAAICSQTLDALRYLHSLKRIHRDIKAGN 155
STE20  G----SKHPNIVNFIDSYVLKGDLWVIMEYMEGGSLTDV-VTHCILTEGQIGAVCRETLSGLEFLHSKGVLHRDIKSDN 744

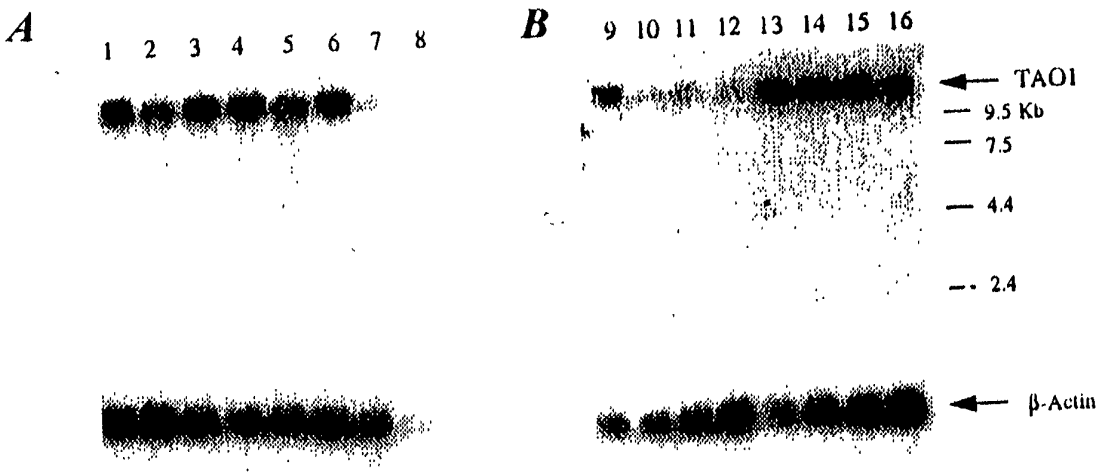
              VII                 VIII                        IX
TAO1   ILLTEPGQVKLADFGSAS----MASPANSFVGTPYWMAPEVILAMDEGQYDGKVDVWSLGITCIELAERKPPLFNMNA 230
TAO2   ILLSEPGLVKLGDFGSAS----IMAPANSFVGTPYWMAPEVILAMDEGQYDGKVDVWSLGITCIELADRKPPLFNMNA 230
ceTAO  ILLSDHAIVKLADFGSAS----LVDPAQTFIGTPFFMAPEVILAMDEGHYTDRADIWSLGITCIELAERRPPLFSMNA 229
STE20  ILLSMEGDIKLTDFGFCAQINELNLKRTTMVGTPYWMAPEVVSRKE---YGPKVDIWSLGIMIIEMIEGEPPYLNETP 819

          X                            XI
TAO1   MSALYHIAQNESPTLQSNEWSDYFRN------FVDSCLQKIPQDRPTSE 273
TAO2   MSALYHIAQNESPALQSGHWSEYFRN------FVDSCLQKIPQDRPTSE 273
ceTAO  MSALYHIAQNDPPTLSPIDTSEQPEWSLEFVQFIDKCLRKPAEERMSAE 279
STE20  LRALYLIATNGTPKLKEPENLSS---SLKK--FLDWCLCVEPEDRASAT 863
```

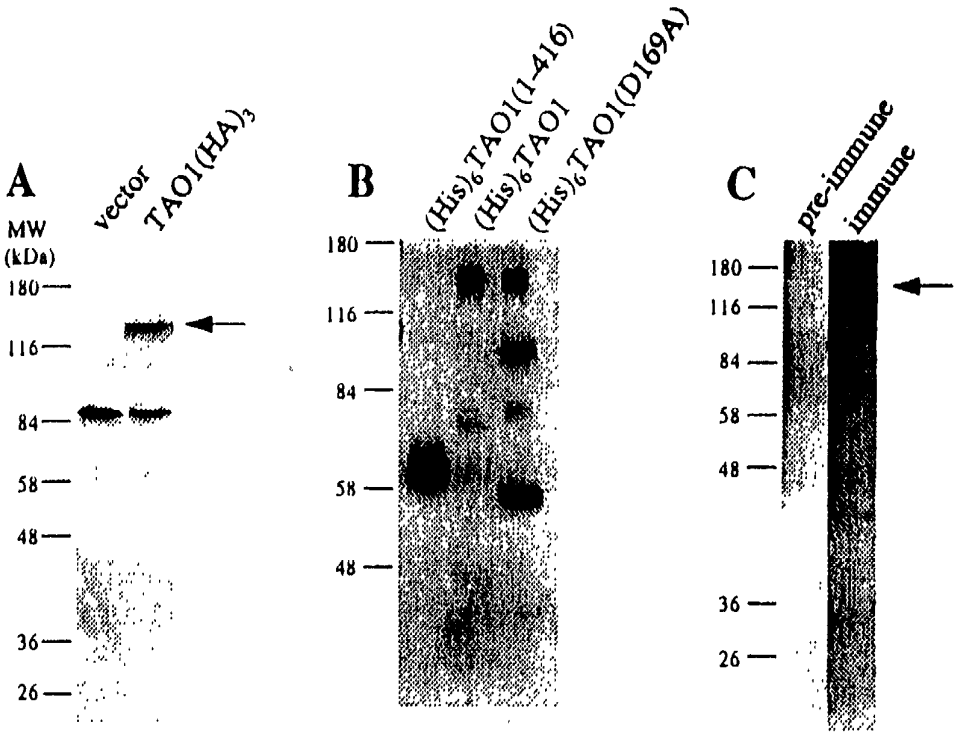
FIG. 2



FIGS. 3A and 3B



FIGS. 4A and 4B



FIGS. 5A-5C

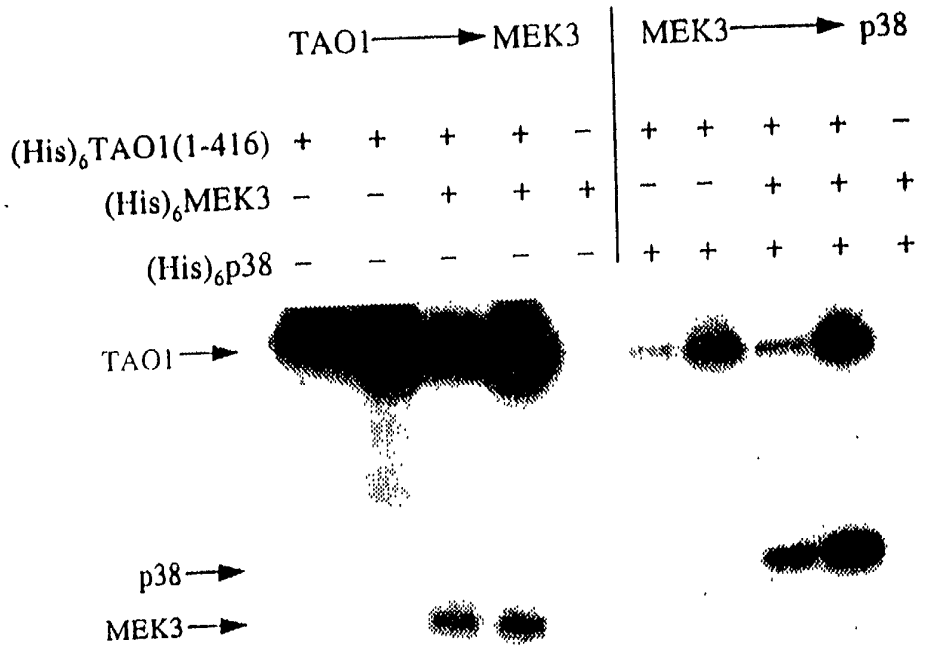

TAO1 → MEK3
MEK3 → p38
(His)6TAO1(1-416) + + + + − + + + + −
(His)6MEK3 − − + + + − − + + +
(His)6p38 − − − − − + + + + +
TAO1 →
p38 →
MEK3 →

FIG. 6

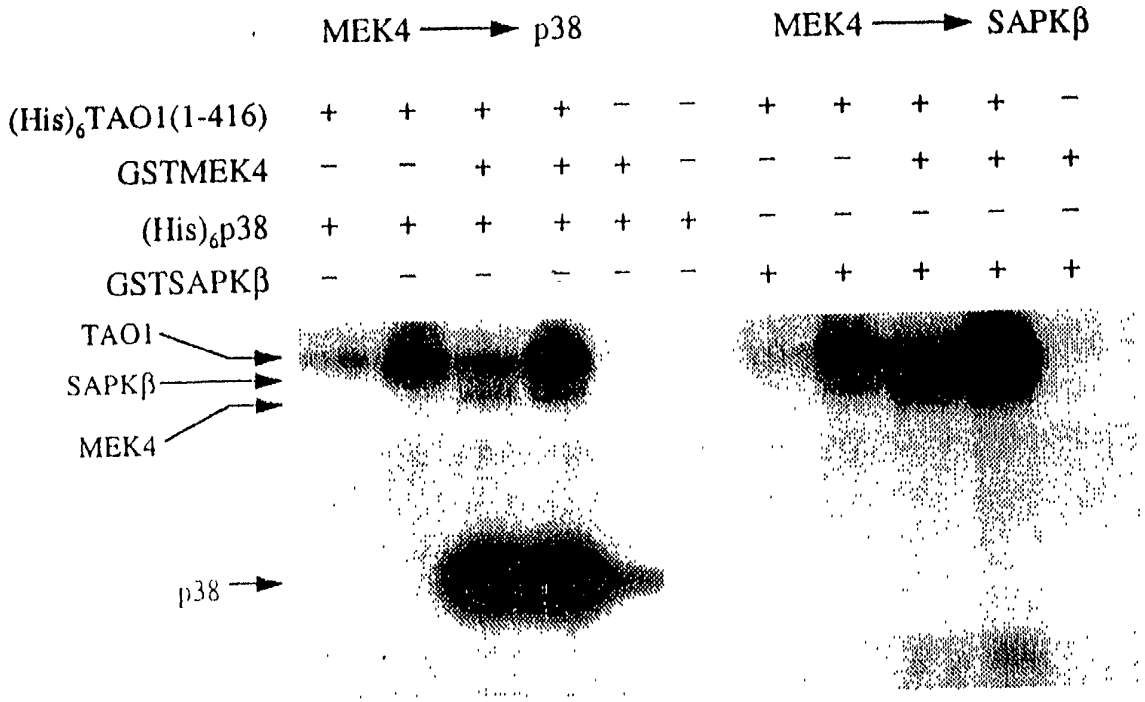

MEK4 → p38
MEK4 → SAPKβ
(His)6TAO1(1-416)
GSTMEK4
(His)6p38
GSTSAPKβ
TAO1
SAPKβ
MEK4
p38

FIG. 7

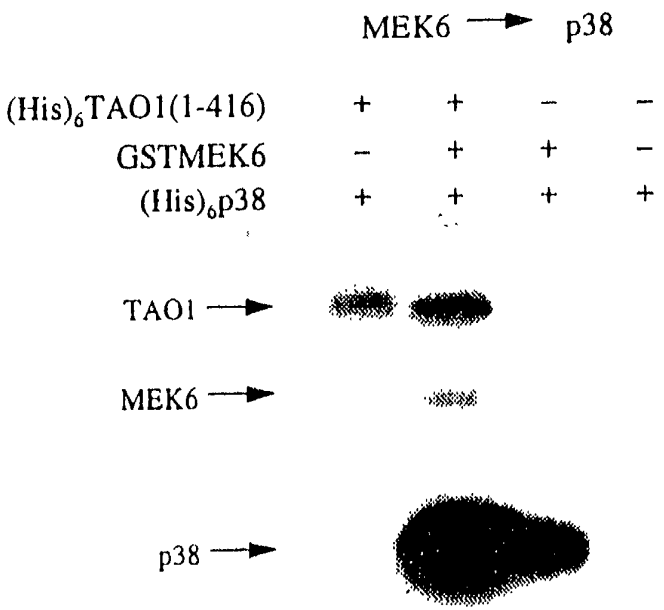


FIG. 8

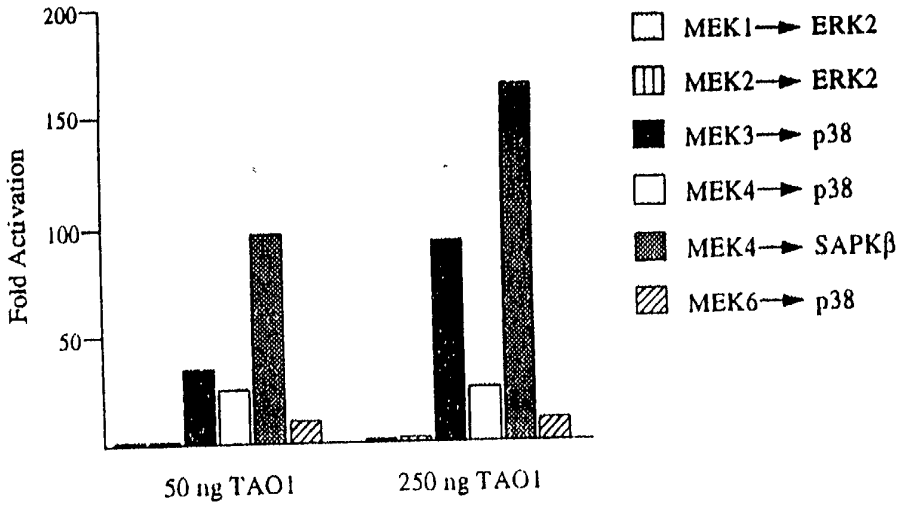

200
150
100
50
Fold Activation
50 ng TAO1
250 ng TAO1
MEK1→ ERK2
MEK2→ ERK2
MEK3→ p38
MEK4→ p38
MEK4→ SAPKβ
MEK6→ p38

FIG. 9

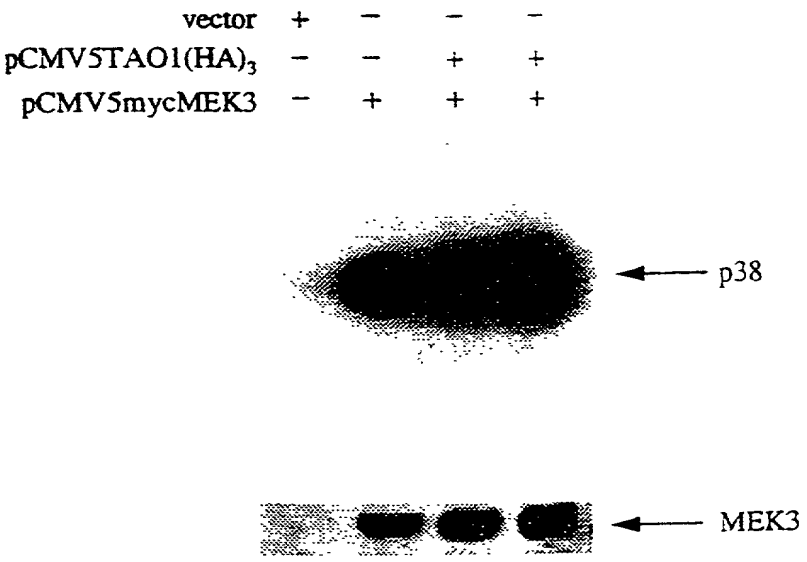


FIG. 10

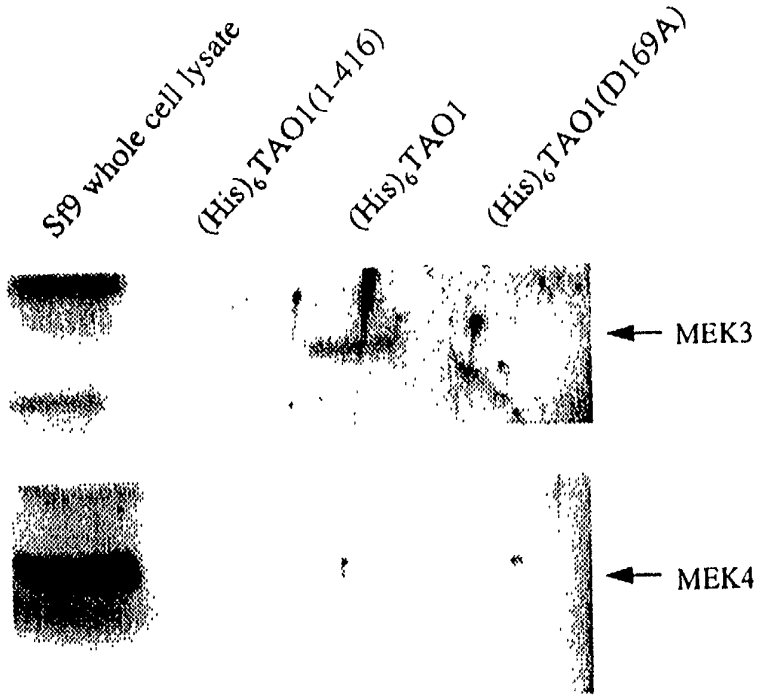


FIG. 11

```
Query: 2341 AGGAATCACCTACTGGAGACTACACCAAAGAGTGAGCACAAAGCTGTTCTGAAAAGACTC 2400
Sbjct:   12 ACGANTCACCAGTTGGAAGTTACTCCAAAGAATGAGCACAAAACAATCTTAAAGACACTG 71

Query: 2401 AAGGAGGAACAGACTCGGAAGTTAGCCATCTTGGCTGAGCAGTATGATCATAGCATTAAT 2460
Sbjct:   72 AAAGATGAGCAGACAAGAAAACTTGCCATTTNGGCAGAGCAGTATGAACAGAGTATAAAT 131

Query: 2461 GAAATGCTCTCCACACAAGCTCTGCGTTTGGATGAAGCACAGGAAGCAGAATGCCAGGTT 2520
Sbjct:  132 GAAATGATGGCCTCTCANGCGTTACGGCTAGATGAGGCTCAAGAAGCAGAATGCCAGGCC 191

Query: 2521 TTGAAGATGCAGCTACAGCAGGAACTGGAGCTGTTGAATGCATATCAGAGCAAAATCAAG 2580
Sbjct:  192 TTGAGGCTACAGCTCCAGCAGGAAATGGAGCTGCTCAACGCCTACCAGAGCAAAATCAAG 251

Query: 2581 ATGCAGGCTGAGGCCCAACATGATCGAGAGCTTCGAGAGCTGGAACAAAGGGTCTCCCTT 2640
Sbjct:  252 ATGCAAACAGAGGCACAACATGAACGTGAGCTCCAGAAGCTAGAGCAGAGAGTGTCTCTG 311

Query: 2641 CGGAGAGCACTCTTAGAACAGAAGATTGAAGAAGAGATGTTGGCTTTGCAGAATGAACGC 2700
Sbjct:  312 CGCAGAGCACACCTTGAGCAGAAGATTGAAGAGGAGCTGGCTGCCCTTCAGAAGGAACGC 371

Query: 2701 ACAGAACGAATACGTAGCCTGCTCGAGCGCCAGGCCAGAGAAATTGAAGCTTTT 2754
Sbjct:  372 AGCGAGAGAATAAAGAACCTATTGGAAAGGCAAGAGCGAGAGATTGGAAACTTT 425
```

FIG. 12

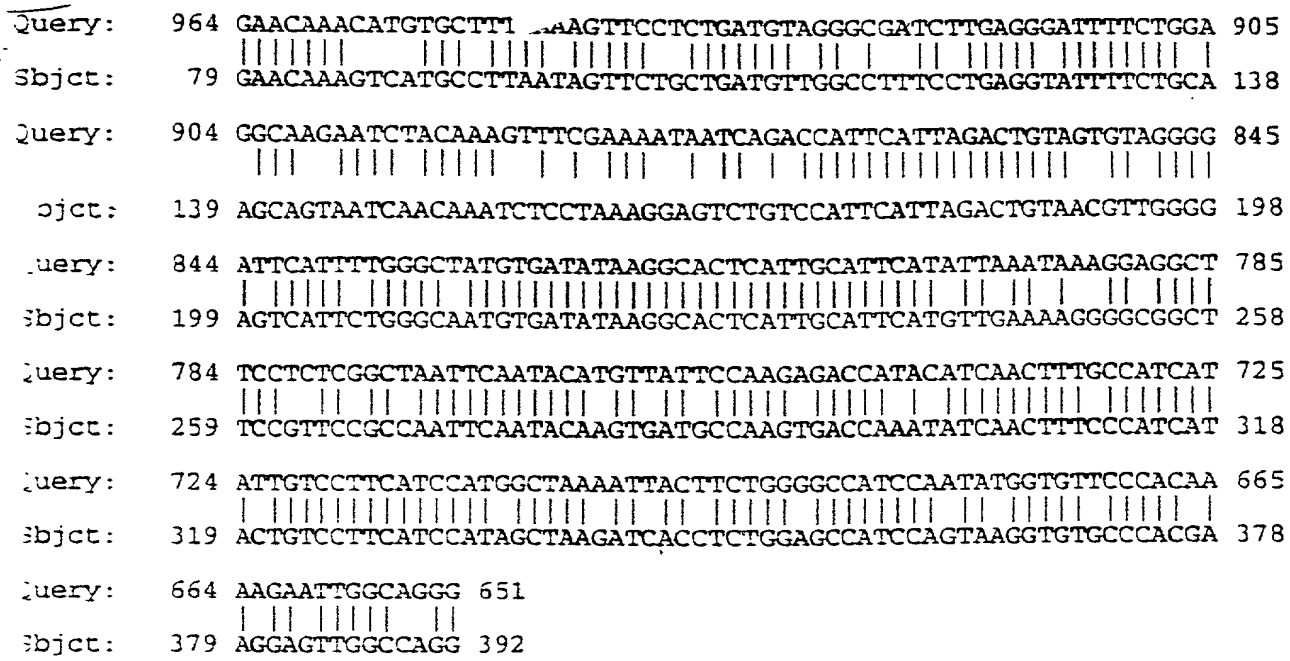

Query: 964 GAACAAACATGTGCTTT AAGTTCCTCTGATGTAGGGCGATCTTGAGGGATTTTCTGGA 905
Sbjct: 79 GAACAAAGTCATGCCTTAATAGTTCTGCTGATGTTGGCCTTTCCTGAGGTATTTTCTGCA 138
Query: 904 GGCAAGAATCTACAAAGTTTCGAAAATAATCAGACCATTCATTAGACTGTAGTGTAGGGG 845
Sbjct: 139 AGCAGTAATCAACAAATCTCCTAAAGGAGTCTGTCCATTCATTAGACTGTAACGTTGGGG 198
Query: 844 ATTCATTTTGGGCTATGTGATATAAGGCACTCATTGCATTCATATTAAATAAAGGAGGCT 785
Sbjct: 199 AGTCATTCTGGGCAATGTGATATAAGGCACTCATTGCATTCATGTTGAAAAGGGGCGGCT 258
Query: 784 TCCTCTCGGCTAATTCAATACATGTTATTCCAAGAGACCATACATCAACTTTGCCATCAT 725
Sbjct: 259 TCCGTTCCGCCAATTCAATACAAGTGATGCCAAGTGACCAAATATCAACTTTCCCATCAT 318
Query: 724 ATTGTCCTTCATCCATGGCTAAAATTACTTCTGGGGCCATCCAATATGGTGTTCCCACAA 665
Sbjct: 319 ACTGTCCTTCATCCATAGCTAAGATCACCTCTGGAGCCATCCAGTAAGGTGTGCCCACGA 378
Query: 664 AAGAATTGGCAGGG 651
Sbjct: 379 AGGAGTTGGCCAGG 392

FIG. 13

```
Query: 2792 ACCATGTTACTAAAACCTAATCTCATGCTTTCAGAGTCAAAAGCTTCAATTTCTCTGGCC 2733

Sbjct:   90 ACCAAATTCCCAAATCCCATTTCTGAGGCTCTCCATGTCAAAAGTTTCAATCTCTCGCTCT 149

Query: 2732 TGGCGCTCGAGCAGGCTACGTATTCGTTCTGTGCGTTCATTCTGCAAAGCCAACATCTCT 2673

Sbjct:  150 TGCCTTTCCAATAGGTTCTTTATTCTCTCGCTGCGTTCCTTCTGAAGGGCAGCCAGCTCC 209

Query: 2672 TCTTCAATCTTCTGTTCTAAGAGTGCTCTCCGAAGGGAGACCCTTTGTTCCAGCTCTCGA 2613

Sbjct:  210 TCTTCAATCTTCTGCTCAAGGTGTGGTCTGCGCAGAGACACTCTCTGCTCTAGCTTCTGG 269

Query: 2612 AGCTCTCGATCATGTTGGGCCTCAGCCTGCATCTTGATTTTGCTCTGATATGCATTCAAC 2553

Sbjct:  270 AGCTCACGTTCATGTTGTGCCTCTGTTNGNATCTTGATTTGGNTCTGGTAGGCGTTGAGC 329

Query: 2552 AGCTCCAGTTCCTGCTGTAGCTGCATCTTCAAAACCTGGCATTCTGCTTCCTGTGCTTCA 2493

Sbjct:  330 AGCTCCATTTCCTGCTGGAGCTGTAGCCTCAAGGCCTGGCATTCTGCTTCTTGAGCCTCA 389

Query: 2492 TCCAAACGCAGAGCTTGTGTGGAGAGCATTTCATTAATGCTATGATCATACTGCTCAGCC 2433

Sbjct:  390 TCTAGCCGTAACGCTTGAGAGGCCATCATTTCATTTATACTCTGTTCATACTGCTCTGCC 449

Query: 2432 AAGATGGCTA 2423

Sbjct:  450 AAAATGGCAA 459
```

FIG. 14

A

```
uery:  2248 CAGCAGCCTAAGAGTTTGAAGTCTAAAGAACTCCAAATAAAAAAGCAGTTTCAGGATACC 2307
            || ||||  || |  || ||| | |  ||| | ||||| ||||| ||||||||||| ||
bjct:     3 CAACAGCAGAAAAACTTAAAGGCCATGGAAATGCAAATTAAAAAACAGTTTCAGGACACT 62

uery:  2308 TGCAAAATTCAAACCAGACAGTACAAAGCATTAAGGAATCACCTACTGGAGACTACACCA 2367
            |||||| | || |||| |||||| |||||| | | |||||||| |||| ||| |||
bjct:    63 TGCAAAGTACAGACCAAACAGTATAAAGCACTCAAGAATCACCAGTTGGAAGTTACTCCA 122

uery:  2368 AAGAGTGAGCACAAAGCTGTTCTGAAAAGACTCAAGGAGGAACAGACTCGGAAGTTAGCC 2427
            |||| |||||||||| |  |  | || | ||| || || || ||||| | ||  | |||
bjct:   123 AAGAATGAGCACAAAACAATCTTAAAGACACTGAAAGATGAGCAGACAAGAAAACTTGCC 182

uery:  2428 ATCTTGGCTG 2437
            || |||||  |
bjct:   183 ATTTTGGCAG 192
```

B

```
uery:  2437 GAGCAGTATGATCATAGCATTAATGAAATGCTCTCCACACAAGCTCTGCGTTTGGATGAA 2496
            |||||||||||| || || || |||||||||| |  || | ||||| | ||  | |||||
bjct:   193 GAGCAGTATGAACAGAGTATAAATGAAATGATGGCCTCTCAAGCGTTACGGCTAGATGAG 252

uery:  2497 GCACA 2501
            || ||
bjct:   253 GCTCA 257
```

FIG. 15A and 15B

```
Query:  2087 ATGAATCCATGCAAGAACTGGAGTTTCGCCACCTCAACACTATTCAGAAGATGCGCTGTG 2146
             | || |||   | ||| || ||||   | || ||  ||||  | ||||| | |||   |
Sbjct:    31 ACGAGTCCCCCCGAGAGCTAGAGTACAGGCAGCTGCACACGTTACAGAAGCTACGCATGG 90

Query:  2147 AGTTGATCAGACTGCAACATCAAACTGAGCTTACTAACCAGCTGGAATACAATAAGAGAA 2206
             |  ||||| |  | || || || || || ||    |||||||||| ||||||||||
Sbjct:    91 ATCTGATCCGTTTACAGCACCAGACGGAACTGGAAAACCAGCTGGAGTACAATAAGAGGC 150

Query:  2207 GGGAACGGGAACTAAGACGGAAACATGTCATGGAAGTTCGACAGCAGCCTAAGAGTTTGA 2266
             | ||| | |||||     | || ||||||||||| |||| || ||||| || |  || |
Sbjct:   151 GAGAAAGAGAACTGCACAGAAAGCATGTCATGGAACTTCGGCAACAGCCAAAAAACTTAA 210

Query:  2267 AGTCTAAAGAACTCCAAATAAAAAAGCAGTTTCAGGATA 2305
             || | |  ||| | ||| | ||||| ||||| ||||| |
Sbjct:   211 AGGCCATGGAANTGCAATTTAAAAAACAGTTCCAGGAAA 249
```

FIG. 16

A

```
Query:  3228 GTGCATATGGTATATTTTATTCGTCTTTGTAAAGCGTTATGTTTTGTGTTTACTAATTGG 3287
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:    31 GTGCATATGGTATATTTNATTCATTTTTGTAAAGCGTTCTGTTTTGTGTTTACTAATTGG 90

Query:  3288 GATGTCATAGTATTTGGCTGCCGGG 3312
             |||||||||||| ||||||||||||
Sbjct:    91 GATGTCATAGTACTTGGCTGCCGGG 115
```

B

```
Query:  3200 CTCACTTGGGTACTACCGGGTGGAAGCTGTGCATATGGTATATTTT 3245
             |||||||||||||||| |||||||||| |  ||   ||| ||
Sbjct:     1 CTCACTTGGGTACTACAGTGTGGAAGCTGAGTGCATATGGTATATT 46
```

FIG. 17A and 17B

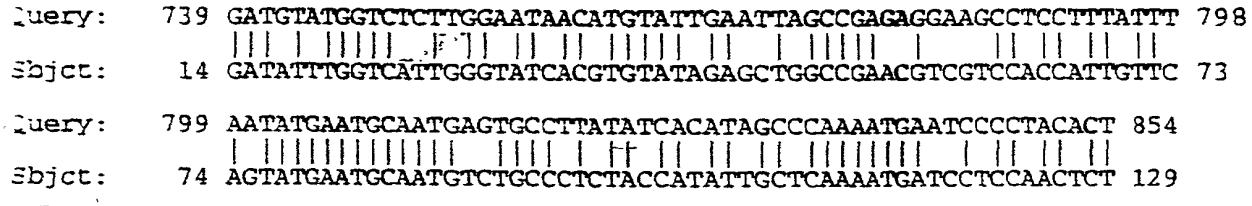

Query: 739 GATGTATGGTCTCTTGGAATAACATGTATTGAATTAGCCGAGAGGAAGCCTCCTTTATTT 798
Sbjct: 14 GATATTTGGTCATTGGGTATCACGTGTATAGAGCTGGCCGAACGTCGTCCACCATTGTTC 73
Query: 799 AATATGAATGCAATGAGTGCCTTATATCACATAGCCCAAAATGAATCCCCTACACT 854
Sbjct: 74 AGTATGAATGCAATGTCTGCCCTCTACCATATTGCTCAAAATGATCCTCCAACTCT 129

FIG. 18

A

```
Query:   526 CTCCAGGGATTAGCTTATTTACATTCTCATACCATGATCCATAGAGATATCAAAGCAGGA 585
             ||  | ||  | | |||| | || ||  |   || ||||||  |||||||||||||| |
Sbjct:   449 CTGAAAGGCCTGGATTATCTGCACTCAGAGCGCAAGATCCACCGAGATATCAAAGCTGCC 508

Query:   586 AATATCCTTCTGACAGAACCAGGCCAAGTGAAACTTGCTGACTTTGGATCTGCTTCCA 643
             ||  | ||  ||  | ||  |  ||  | |||||  | || |||||  ||   |||  ||
Sbjct:   509 AACGTGCTGCTCTCGGAGCAGGGTGATGTGAAGATGGCAGACTTCGGTGTGGCTGGCA 566
```

B

```
Query:   187 GATCCGGAAAAACTCTTCACAGATCTCAGAGAAATCGGCCATGGGAGCTTTGGAGCAGTT 246
             || || ||  ||||||||||  | ||       || ||| | ||   |||||  |  ||
Sbjct:   119 GACCCAGAGGAACTCTTCACCAAGCTTGACCGCATTGGCAAAGGCTCATTTGGGGAGGTG 178

Query:   247 TATTTTGCACGAGATGTGCGTACTAATGAAGTGGTGGCCATCAAGAAAAT 296
             ||    |    ||    |  || || ||||||||||||||||||||  ||
Sbjct:   179 TACAAGGGGATCGACAACCACACCAAGGAAGTGGTGGCCATCAAGATCAT 228
```

FIG. 19A and 19B

```
Query: 866 TCATTAGACTGTAGTGTAGGGGATTCATTTTGGGCTATGTGATATAAGGCACTCATTGCA 807
           |||  |  ||| ||    || | | ||||   ||||||  |||| || || || | || |
Sbjct: 100 TCAGGATTCTGGAGCTCTGGAGTTCCATTAGTGGCTATCAGATACAATGCCCTGAGTGGA 159

Query: 806 TTCATATTAAATAAAGGAGGCTTCCTCTCGGCTAATTCAATACATGTTATTCCAAGAGAC 747
           ||   |||||   |||| || |  |  ||  | | ||||||     | ||||||||||||
Sbjct: 160 TTTTCATTAAGGTAAGGGGGTTCACCTTCCACCATTTCAATTGCCATAATTCCAAGAGAC 219

Query: 746 CATACATCAACTTT 733
           || | |||||||||
Sbjct: 220 CAGATATCAACTTT 233
```

FIG. 20

EXPRESS MAIL NO. EL 487806495US
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Melanie Cobb, Michele Hutchison, Zhu Chen and Kevin Berman
Filed	:	October 10, 2000
For	:	TAO PROTEIN KINASE POLYPEPTIDES AND METHODS OF USE THEREFOR

Docket No.	:	860098.421C1
Date	:	October 10, 2000

Box Patent Application
Assistant Commissioner for Patents
Washington, DC 20231

DECLARATION

Sir:

I, Bonita R. Gladden, in accordance with 37 C.F.R. § 1.821(f) do hereby declare that, to the best of my knowledge, the content of the paper entitled "Sequence Listing" and the computer readable copy contained within the floppy disk are the same.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated this 10th day of October, 2000.

Bonita R. Gladden
Legal Assistant

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Seattle, WA 98104-7092
Phone: (206) 622-4900
Fax: (206) 682-6031
Wpn/860098/421C1Forms/Seq Dec

SEQUENCE LISTING

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<110> Cobb, Melanie
      Hutchison, Michele
      Chen, Zhu
      Berman, Kevin

<120> TAO PROTEIN KINASE POLYPEPTIDES AND
  METHODS OF USE THEREFOR

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tgaaccaagc tttgggatag cagtataaaa ttagaatcag acagctgact gctcagcagg    120
atg cca tca act aac aga gca ggc agt cta aag gac cct gaa atc gca      168
Met Pro Ser Thr Asn Arg Ala Gly Ser Leu Lys Asp Pro Glu Ile Ala
 1               5                  10                  15

gag ctc ttc ttc aaa gaa gat ccg gaa aaa ctc ttc aca gat ctc aga      216
Glu Leu Phe Phe Lys Glu Asp Pro Glu Lys Leu Phe Thr Asp Leu Arg
            20                  25                  30

gaa atc ggc cat ggg agc ttt gga gca gtt tat ttt gca cga gat gtg      264
Glu Ile Gly His Gly Ser Phe Gly Ala Val Tyr Phe Ala Arg Asp Val
        35                  40                  45

cgt act aat gaa gtg gtg gcc atc aag aaa atg tct tat agt gga aag      312
Arg Thr Asn Glu Val Val Ala Ile Lys Lys Met Ser Tyr Ser Gly Lys
    50                  55                  60

cag tct act gag aaa tgg cag gat att att aag gaa gtc aag ttt cta      360
Gln Ser Thr Glu Lys Trp Gln Asp Ile Ile Lys Glu Val Lys Phe Leu
 65                  70                  75                  80

caa aga ata aaa cat ccc aac agt ata gaa tac aaa ggc tgc tat tta      408
Gln Arg Ile Lys His Pro Asn Ser Ile Glu Tyr Lys Gly Cys Tyr Leu
                85                  90                  95

cgt gaa cac aca gca tgg ctt gta atg gaa tat tgt tta gga tct gct      456
Arg Glu His Thr Ala Trp Leu Val Met Glu Tyr Cys Leu Gly Ser Ala
```

```
        100                 105                 110

tcg gat tta cta gaa gtt cat aaa aag cca tta caa gaa gtg gaa ata      504
Ser Asp Leu Leu Glu Val His Lys Lys Pro Leu Gln Glu Val Glu Ile
            115                 120                 125

gca gca att aca cat ggt gct ctc cag gga tta gct tat tta cat tct      552
Ala Ala Ile Thr His Gly Ala Leu Gln Gly Leu Ala Tyr Leu His Ser
    130                 135                 140

cat acc atg atc cat aga gat atc aaa gca gga aat atc ctt ctg aca      600
His Thr Met Ile His Arg Asp Ile Lys Ala Gly Asn Ile Leu Leu Thr
145                 150                 155                 160

gaa cca ggc caa gtg aaa ctt gct gac ttt gga tct gct tcc atg gcc      648
Glu Pro Gly Gln Val Lys Leu Ala Asp Phe Gly Ser Ala Ser Met Ala
                165                 170                 175

tcc cct gcc aat tct ttt gtg gga aca cca tat tgg atg gcc cca gaa      696
Ser Pro Ala Asn Ser Phe Val Gly Thr Pro Tyr Trp Met Ala Pro Glu
            180                 185                 190

gta att tta gcc atg gat gaa gga caa tat gat ggc aaa gtt gat gta      744
Val Ile Leu Ala Met Asp Glu Gly Gln Tyr Asp Gly Lys Val Asp Val
        195                 200                 205

tgg tct ctt gga ata aca tgt att gaa tta gcc gag agg aag cct cct      792
Trp Ser Leu Gly Ile Thr Cys Ile Glu Leu Ala Glu Arg Lys Pro Pro
    210                 215                 220

tta ttt aat atg aat gca atg agt gcc tta tat cac ata gcc caa aat      840
Leu Phe Asn Met Asn Ala Met Ser Ala Leu Tyr His Ile Ala Gln Asn
225                 230                 235                 240

gaa tcc cct aca cta cag tct aat gaa tgg tct gat tat ttt cga aac      888
Glu Ser Pro Thr Leu Gln Ser Asn Glu Trp Ser Asp Tyr Phe Arg Asn
                245                 250                 255

ttt gta gat tct tgc ctc cag aaa atc cct caa gat cgc cct aca tca      936
Phe Val Asp Ser Cys Leu Gln Lys Ile Pro Gln Asp Arg Pro Thr Ser
            260                 265                 270

gag gaa ctt tta aag cac atg ttt gtt ctt cga gag cgc cct gaa aca      984
Glu Glu Leu Leu Lys His Met Phe Val Leu Arg Glu Arg Pro Glu Thr
        275                 280                 285

gtg tta ata gat ctt att caa agg aca aag gat gca gta aga gag ctg     1032
Val Leu Ile Asp Leu Ile Gln Arg Thr Lys Asp Ala Val Arg Glu Leu
    290                 295                 300

gac aat cta caa tat cga aag atg aag aaa ctc ctt ttc cag gag gca     1080
Asp Asn Leu Gln Tyr Arg Lys Met Lys Lys Leu Leu Phe Gln Glu Ala
305                 310                 315                 320

cat aat gga cca gca gta gaa gca cag gaa gaa gag gag gag caa gat     1128
His Asn Gly Pro Ala Val Glu Ala Gln Glu Glu Glu Glu Glu Gln Asp
                325                 330                 335
```

```
cat ggt ggt ggc cgg aca gga aca gta aat agt gtt gga agc aat cag      1176
His Gly Gly Gly Arg Thr Gly Thr Val Asn Ser Val Gly Ser Asn Gln
                340                 345                 350

tct atc ccc agt atg tct atc agt gcc agt agc caa agc agc agt gtt      1224
Ser Ile Pro Ser Met Ser Ile Ser Ala Ser Ser Gln Ser Ser Ser Val
        355                 360                 365

aat agt ctt cca gat gca tcg gat gac aag agt gag cta gac atg atg      1272
Asn Ser Leu Pro Asp Ala Ser Asp Asp Lys Ser Glu Leu Asp Met Met
    370                 375                 380

gag gga gac cat aca gtg atg tct aac agt tct gtc atc cac tta aaa      1320
Glu Gly Asp His Thr Val Met Ser Asn Ser Ser Val Ile His Leu Lys
385                 390                 395                 400

cct gag gag gaa aat tac caa gaa gaa gga gat cct aga aca aga gca      1368
Pro Glu Glu Glu Asn Tyr Gln Glu Glu Gly Asp Pro Arg Thr Arg Ala
                405                 410                 415

tca gct cca cag tct cca cct caa gtg tct cgt cac aaa tca cat tat      1416
Ser Ala Pro Gln Ser Pro Pro Gln Val Ser Arg His Lys Ser His Tyr
            420                 425                 430

cgt aat aga gaa cac ttt gca act ata cga aca gca tca ctg gtt aca      1464
Arg Asn Arg Glu His Phe Ala Thr Ile Arg Thr Ala Ser Leu Val Thr
        435                 440                 445

aga cag atg caa gaa cat gag cag gac tct gaa ctt aga gaa cag atg      1512
Arg Gln Met Gln Glu His Glu Gln Asp Ser Glu Leu Arg Glu Gln Met
    450                 455                 460

tct ggt tat aag cgg atg agg cga cag cat cag aag cag ctg atg act      1560
Ser Gly Tyr Lys Arg Met Arg Arg Gln His Gln Lys Gln Leu Met Thr
465                 470                 475                 480

ctg gaa aat aaa ctg aag gca gaa atg gac gaa cat cgg ctc aga tta      1608
Leu Glu Asn Lys Leu Lys Ala Glu Met Asp Glu His Arg Leu Arg Leu
                485                 490                 495

gac aaa gat ctt gaa act cag cgc aac aat ttc gct gca gaa atg gag      1656
Asp Lys Asp Leu Glu Thr Gln Arg Asn Asn Phe Ala Ala Glu Met Glu
            500                 505                 510

aaa ctt att aag aaa cac caa gct tct atg gaa aaa gag gct aaa gtg      1704
Lys Leu Ile Lys Lys His Gln Ala Ser Met Glu Lys Glu Ala Lys Val
        515                 520                 525

atg gcc aac gag gag aaa aaa ttc caa caa cac att cag gct caa cag      1752
Met Ala Asn Glu Glu Lys Lys Phe Gln Gln His Ile Gln Ala Gln Gln
    530                 535                 540

aag aaa gaa ctg aat agc ttt ttg gag tct caa aaa aga gaa tat aaa      1800
Lys Lys Glu Leu Asn Ser Phe Leu Glu Ser Gln Lys Arg Glu Tyr Lys
545                 550                 555                 560

ctt cga aaa gag cag ctt aag gag gag ctg aat gaa aac cag agc aca      1848
Leu Arg Lys Glu Gln Leu Lys Glu Glu Leu Asn Glu Asn Gln Ser Thr
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            565                 570                 575

cct aaa aaa gaa aag cag gaa tgg ctt tca aag cag aag gag aat att      1896
Pro Lys Lys Glu Lys Gln Glu Trp Leu Ser Lys Gln Lys Glu Asn Ile
            580                 585                 590

caa cat ttt cag gca gaa gaa gaa gct aat ctt ctt cga cgt caa agg      1944
Gln His Phe Gln Ala Glu Glu Glu Ala Asn Leu Leu Arg Arg Gln Arg
        595                 600                 605

cag tat cta gag cta gaa tgt cgt cgc ttc aaa aga aga atg tta ctt      1992
Gln Tyr Leu Glu Leu Glu Cys Arg Arg Phe Lys Arg Arg Met Leu Leu
    610                 615                 620

ggt cgg cat aac ttg gaa cag gac ctt gtc agg gag gag tta aac aaa      2040
Gly Arg His Asn Leu Glu Gln Asp Leu Val Arg Glu Glu Leu Asn Lys
625                 630                 635                 640

agg cag act cag aag gac tta gaa cat gca atg tta ctg cga cag cat      2088
Arg Gln Thr Gln Lys Asp Leu Glu His Ala Met Leu Leu Arg Gln His
                645                 650                 655

gaa tcc atg caa gaa ctg gag ttt cgc cac ctc aac act att cag aag      2136
Glu Ser Met Gln Glu Leu Glu Phe Arg His Leu Asn Thr Ile Gln Lys
            660                 665                 670

atg cgc tgt gag ttg atc aga ctg caa cat caa act gag ctt act aac      2184
Met Arg Cys Glu Leu Ile Arg Leu Gln His Gln Thr Glu Leu Thr Asn
        675                 680                 685

cag ctg gaa tac aat aag aga agg gaa cgg gaa cta aga cgg aaa cat      2232
Gln Leu Glu Tyr Asn Lys Arg Arg Glu Arg Glu Leu Arg Arg Lys His
    690                 695                 700

gtc atg gaa gtt cga cag cag cct aag agt ttg aag tct aaa gaa ctc      2280
Val Met Glu Val Arg Gln Gln Pro Lys Ser Leu Lys Ser Lys Glu Leu
705                 710                 715                 720

caa ata aaa aag cag ttt cag gat acc tgc aaa att caa acc aga cag      2328
Gln Ile Lys Lys Gln Phe Gln Asp Thr Cys Lys Ile Gln Thr Arg Gln
                725                 730                 735

tac aaa gca tta agg aat cac cta ctg gag act aca cca aag agt gag      2376
Tyr Lys Ala Leu Arg Asn His Leu Leu Glu Thr Thr Pro Lys Ser Glu
            740                 745                 750

cac aaa gct gtt ctg aaa aga ctc aag gag gaa cag act cgg aag tta      2424
His Lys Ala Val Leu Lys Arg Leu Lys Glu Glu Gln Thr Arg Lys Leu
        755                 760                 765

gcc atc ttg gct gag cag tat gat cat agc att aat gaa atg ctc tcc      2472
Ala Ile Leu Ala Glu Gln Tyr Asp His Ser Ile Asn Glu Met Leu Ser
    770                 775                 780

aca caa gct ctg cgt ttg gat gaa gca cag gaa gca gaa tgc cag gtt      2520
Thr Gln Ala Leu Arg Leu Asp Glu Ala Gln Glu Ala Glu Cys Gln Val
785                 790                 795                 800
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ttg aag atg cag cta cag cag gaa ctg gag ctg ttg aat gca tat cag 2568
Leu Lys Met Gln Leu Gln Gln Glu Leu Glu Leu Leu Asn Ala Tyr Gln
805 810 815

agc aaa atc aag atg cag gct gag gcc caa cat gat cga gag ctt cga 2616
Ser Lys Ile Lys Met Gln Ala Glu Ala Gln His Asp Arg Glu Leu Arg
820 825 830

gag ctg gaa caa agg gtc tcc ctt cgg aga gca ctc tta gaa cag aag 2664
Glu Leu Glu Gln Arg Val Ser Leu Arg Arg Ala Leu Leu Glu Gln Lys
835 840 845

att gaa gaa gag atg ttg gct ttg cag aat gaa cgc aca gaa cga ata 2712
Ile Glu Glu Glu Met Leu Ala Leu Gln Asn Glu Arg Thr Glu Arg Ile
850 855 860

cgt agc ctg ctc gag cgc cag gcc aga gaa att gaa gct ttt gac tct 2760
Arg Ser Leu Leu Glu Arg Gln Ala Arg Glu Ile Glu Ala Phe Asp Ser
865 870 875 880

gaa agc atg aga tta ggt ttt agt aac atg gtc ctt tct aat ctc tcc 2808
Glu Ser Met Arg Leu Gly Phe Ser Asn Met Val Leu Ser Asn Leu Ser
885 890 895

cct gag gca ttc agc cac agc tac cca gga gct tct agc tgg tct cac 2856
Pro Glu Ala Phe Ser His Ser Tyr Pro Gly Ala Ser Ser Trp Ser His
900 905 910

aat cct act ggg ggt tca gga cct cac tgg ggt cat ccc atg ggt ggc 2904
Asn Pro Thr Gly Gly Ser Gly Pro His Trp Gly His Pro Met Gly Gly
915 920 925

aca cca caa gct tgg ggt cat ccg atg caa ggc gga ccc caa cca tgg 2952
Thr Pro Gln Ala Trp Gly His Pro Met Gln Gly Gly Pro Gln Pro Trp
930 935 940

ggt cac ccc tca ggg cca atg caa ggg gta cct cga ggt agc agt ata 3000
Gly His Pro Ser Gly Pro Met Gln Gly Val Pro Arg Gly Ser Ser Ile
945 950 955 960

gga gtc cgc aat agc ccc cag gct ctg agg cgg aca gct tct ggg gga 3048
Gly Val Arg Asn Ser Pro Gln Ala Leu Arg Arg Thr Ala Ser Gly Gly
965 970 975

cgg acg gaa cag ggc atg agc aga agc acg agt gtc act tca caa ata 3096
Arg Thr Glu Gln Gly Met Ser Arg Ser Thr Ser Val Thr Ser Gln Ile
980 985 990

tcc aat ggg tca cac atg tct tac aca taataattga aagtggcaat 3143
Ser Asn Gly Ser His Met Ser Tyr Thr
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cttgggtact accgggtgga agctgtgcat atggtatatt ttattcgtct ttgtaaagcg 3263
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Glu Ile Gly His Gly Ser Phe Gly Ala Val Tyr Phe Ala Arg Asp Val
        35                  40                  45
Arg Thr Asn Glu Val Val Ala Ile Lys Lys Met Ser Tyr Ser Gly Lys
    50                  55                  60
Gln Ser Thr Glu Lys Trp Gln Asp Ile Ile Lys Glu Val Lys Phe Leu
65                  70                  75                  80
Gln Arg Ile Lys His Pro Asn Ser Ile Glu Tyr Lys Gly Cys Tyr Leu
                85                  90                  95
Arg Glu His Thr Ala Trp Leu Val Met Glu Tyr Cys Leu Gly Ser Ala
            100                 105                 110
Ser Asp Leu Leu Glu Val His Lys Lys Pro Leu Gln Glu Val Glu Ile
        115                 120                 125
Ala Ala Ile Thr His Gly Ala Leu Gln Gly Leu Ala Tyr Leu His Ser
    130                 135                 140
His Thr Met Ile His Arg Asp Ile Lys Ala Gly Asn Ile Leu Leu Thr
145                 150                 155                 160
Glu Pro Gly Gln Val Lys Leu Ala Asp Phe Gly Ser Ala Ser Met Ala
                165                 170                 175
Ser Pro Ala Asn Ser Phe Val Gly Thr Pro Tyr Trp Met Ala Pro Glu
            180                 185                 190
Val Ile Leu Ala Met Asp Glu Gly Gln Tyr Asp Gly Lys Val Asp Val
        195                 200                 205
Trp Ser Leu Gly Ile Thr Cys Ile Glu Leu Ala Glu Arg Lys Pro Pro
    210                 215                 220
Leu Phe Asn Met Asn Ala Met Ser Ala Leu Tyr His Ile Ala Gln Asn
225                 230                 235                 240
Glu Ser Pro Thr Leu Gln Ser Asn Glu Trp Ser Asp Tyr Phe Arg Asn
                245                 250                 255
Phe Val Asp Ser Cys Leu Gln Lys Ile Pro Gln Asp Arg Pro Thr Ser
            260                 265                 270
Glu Glu Leu Leu Lys His Met Phe Val Leu Arg Glu Arg Pro Glu Thr
        275                 280                 285
Val Leu Ile Asp Leu Ile Gln Arg Thr Lys Asp Ala Val Arg Glu Leu
    290                 295                 300
Asp Asn Leu Gln Tyr Arg Lys Met Lys Lys Leu Leu Phe Gln Glu Ala
305                 310                 315                 320
His Asn Gly Pro Ala Val Glu Ala Gln Glu Glu Glu Glu Glu Gln Asp
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His Gly Gly Gly Arg Thr Gly Thr Val Asn Ser Val Gly Ser Asn Gln
            340                 345                 350
Ser Ile Pro Ser Met Ser Ile Ser Ala Ser Ser Gln Ser Ser Ser Val
        355                 360                 365
Asn Ser Leu Pro Asp Ala Ser Asp Asp Lys Ser Glu Leu Asp Met Met
    370                 375                 380
Glu Gly Asp His Thr Val Met Ser Asn Ser Ser Val Ile His Leu Lys
385                 390                 395                 400
Pro Glu Glu Glu Asn Tyr Gln Glu Glu Gly Asp Pro Arg Thr Arg Ala
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Ser Ala Pro Gln Ser Pro Pro Gln Val Ser Arg His Lys Ser His Tyr
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Arg Asn Arg Glu His Phe Ala Thr Ile Arg Thr Ala Ser Leu Val Thr
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Arg Gln Met Gln Glu His Glu Gln Asp Ser Glu Leu Arg Glu Gln Met
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Ser Gly Tyr Lys Arg Met Arg Arg Gln His Gln Lys Gln Leu Met Thr
465                 470                 475                 480
Leu Glu Asn Lys Leu Lys Ala Glu Met Asp Glu His Arg Leu Arg Leu
                485                 490                 495
Asp Lys Asp Leu Glu Thr Gln Arg Asn Asn Phe Ala Ala Glu Met Glu
            500                 505                 510
Lys Leu Ile Lys Lys His Gln Ala Ser Met Glu Lys Glu Ala Lys Val
        515                 520                 525
Met Ala Asn Glu Glu Lys Lys Phe Gln Gln His Ile Gln Ala Gln Gln
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Lys Lys Glu Leu Asn Ser Phe Leu Glu Ser Gln Lys Arg Glu Tyr Lys
545                 550                 555                 560
Leu Arg Lys Glu Gln Leu Lys Glu Glu Leu Asn Glu Asn Gln Ser Thr
                565                 570                 575
Pro Lys Lys Glu Lys Gln Glu Trp Leu Ser Lys Gln Lys Glu Asn Ile
            580                 585                 590
Gln His Phe Gln Ala Glu Glu Glu Ala Asn Leu Leu Arg Arg Gln Arg
        595                 600                 605
Gln Tyr Leu Glu Leu Glu Cys Arg Arg Phe Lys Arg Arg Met Leu Leu
    610                 615                 620
Gly Arg His Asn Leu Glu Gln Asp Leu Val Arg Glu Glu Leu Asn Lys
625                 630                 635                 640
Arg Gln Thr Gln Lys Asp Leu Glu His Ala Met Leu Leu Arg Gln His
                645                 650                 655
Glu Ser Met Gln Glu Leu Glu Phe Arg His Leu Asn Thr Ile Gln Lys
            660                 665                 670
Met Arg Cys Glu Leu Ile Arg Leu Gln His Gln Thr Glu Leu Thr Asn
        675                 680                 685
Gln Leu Glu Tyr Asn Lys Arg Arg Glu Arg Glu Leu Arg Arg Lys His
    690                 695                 700
Val Met Glu Val Arg Gln Gln Pro Lys Ser Leu Lys Ser Lys Glu Leu
705                 710                 715                 720
Gln Ile Lys Lys Gln Phe Gln Asp Thr Cys Lys Ile Gln Thr Arg Gln
                725                 730                 735
Tyr Lys Ala Leu Arg Asn His Leu Leu Glu Thr Thr Pro Lys Ser Glu
            740                 745                 750
His Lys Ala Val Leu Lys Arg Leu Lys Glu Glu Gln Thr Arg Lys Leu
        755                 760                 765
Ala Ile Leu Ala Glu Gln Tyr Asp His Ser Ile Asn Glu Met Leu Ser
    770                 775                 780
Thr Gln Ala Leu Arg Leu Asp Glu Ala Gln Glu Ala Glu Cys Gln Val
785                 790                 795                 800
Leu Lys Met Gln Leu Gln Gln Glu Leu Glu Leu Leu Asn Ala Tyr Gln
                805                 810                 815
Ser Lys Ile Lys Met Gln Ala Glu Ala Gln His Asp Arg Glu Leu Arg
            820                 825                 830
Glu Leu Glu Gln Arg Val Ser Leu Arg Arg Ala Leu Leu Glu Gln Lys
        835                 840                 845
Ile Glu Glu Glu Met Leu Ala Leu Gln Asn Glu Arg Thr Glu Arg Ile
    850                 855                 860
Arg Ser Leu Leu Glu Arg Gln Ala Arg Glu Ile Glu Ala Phe Asp Ser
865                 870                 875                 880
Glu Ser Met Arg Leu Gly Phe Ser Asn Met Val Leu Ser Asn Leu Ser
                885                 890                 895
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Pro Glu Ala Phe Ser His Ser Tyr Pro Gly Ala Ser Ser Trp Ser His
900 905 910
Asn Pro Thr Gly Gly Ser Gly Pro His Trp Gly His Pro Met Gly Gly
915 920 925
Thr Pro Gln Ala Trp Gly His Pro Met Gln Gly Gly Pro Gln Pro Trp
930 935 940
Gly His Pro Ser Gly Pro Met Gln Gly Val Pro Arg Gly Ser Ser Ile
945 950 955 960
Gly Val Arg Asn Ser Pro Gln Ala Leu Arg Arg Thr Ala Ser Gly Gly
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980 985 990
Ser Asn Gly Ser His Met Ser Tyr Thr
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agcggaggcg ctggggcacc atagtgaccc ctaccaggca agatcccaat ttcagggccc 180
ccaggggcca tc atg cca gct ggg ggc cgg gcc ggg agc ctg aag gac cct 231
Met Pro Ala Gly Gly Arg Ala Gly Ser Leu Lys Asp Pro
1 5 10

gat gta gct gag ctc ttc ttc aaa gat gac cct gag aag ctt ttc tct 279
Asp Val Ala Glu Leu Phe Phe Lys Asp Asp Pro Glu Lys Leu Phe Ser
15 20 25

gac ctc cgg gaa att ggc cat ggc agt ttt gga gct gtg tac ttt gcc 327
Asp Leu Arg Glu Ile Gly His Gly Ser Phe Gly Ala Val Tyr Phe Ala
30 35 40 45

cgg gat gtc cgg aac agt gag gtg gtg gcc atc aag aag atg tcc tat 375
Arg Asp Val Arg Asn Ser Glu Val Val Ala Ile Lys Lys Met Ser Tyr
50 55 60

agt ggg aag caa tca aat gag aaa tgg cag gat atc atc aag gag gtg 423
Ser Gly Lys Gln Ser Asn Glu Lys Trp Gln Asp Ile Ile Lys Glu Val
65 70 75

cgg ttc tta cag aag cta cgg cat cct aat acc att cag tac cgg ggc 471
Arg Phe Leu Gln Lys Leu Arg His Pro Asn Thr Ile Gln Tyr Arg Gly
80 85 90

tgt tac ctg agg gag cac aca gct tgg ctg gtg atg gag tat tgc ctg 519
Cys Tyr Leu Arg Glu His Thr Ala Trp Leu Val Met Glu Tyr Cys Leu
95 100 105

ggt tca gct tct gat ctt ctc gaa gtg cac aag aag ccg ctg cag gag 567
Gly Ser Ala Ser Asp Leu Leu Glu Val His Lys Lys Pro Leu Gln Glu

110 115 120 125

gta gag att gca gct gtg acc cat ggt gcg ctt cag ggc ctg gcc tat 615
Val Glu Ile Ala Ala Val Thr His Gly Ala Leu Gln Gly Leu Ala Tyr
130 135 140

cta cat tca cac aac atg atc cat aga gat gtg aag gct ggg aac atc 663
Leu His Ser His Asn Met Ile His Arg Asp Val Lys Ala Gly Asn Ile
145 150 155

ttg ctg tca gaa cca ggc ttg gtg aaa ctg ggg gac ttt ggc tcc gca 711
Leu Leu Ser Glu Pro Gly Leu Val Lys Leu Gly Asp Phe Gly Ser Ala
160 165 170

tcc atc atg gca cct gcc aac tca ttt gtg ggc act cca tac tgg atg 759
Ser Ile Met Ala Pro Ala Asn Ser Phe Val Gly Thr Pro Tyr Trp Met
175 180 185

gct cca gag gtg atc cta gcc atg gat gag gga caa tat gat ggc aaa 807
Ala Pro Glu Val Ile Leu Ala Met Asp Glu Gly Gln Tyr Asp Gly Lys
190 195 200 205

gtg gat gtc tgg tcc ttg ggg ata acc tgt att gag cta gcg gag cgg 855
Val Asp Val Trp Ser Leu Gly Ile Thr Cys Ile Glu Leu Ala Glu Arg
210 215 220

aag cca cca ctg ttt aac atg aat gca atg agt gcc tta tac cac att 903
Lys Pro Pro Leu Phe Asn Met Asn Ala Met Ser Ala Leu Tyr His Ile
225 230 235

gca cag aat gaa tcc cct gct ctc cag tca gga cac tgg tct gag tac 951
Ala Gln Asn Glu Ser Pro Ala Leu Gln Ser Gly His Trp Ser Glu Tyr
240 245 250

ttc cgg aat ttt gtt gac tcc tgt ctt cag aaa atc cct caa gac aga 999
Phe Arg Asn Phe Val Asp Ser Cys Leu Gln Lys Ile Pro Gln Asp Arg
255 260 265

cca acc tca gag gtt ctt ttg aag cac cgc ttt gtg ctc cgg gag cgg 1047
Pro Thr Ser Glu Val Leu Leu Lys His Arg Phe Val Leu Arg Glu Arg
270 275 280 285

cca ccc aca gtc atc atg gac cta att cag agg acc aag gat gct gta 1095
Pro Pro Thr Val Ile Met Asp Leu Ile Gln Arg Thr Lys Asp Ala Val
290 295 300

cgg gaa cta gat aac ctg cag tac cga aag atg aag aag ata cta ttc 1143
Arg Glu Leu Asp Asn Leu Gln Tyr Arg Lys Met Lys Lys Ile Leu Phe
305 310 315

caa gag gca ccc aat ggc cct ggt gct gag gcc cca gag gaa gag gag 1191
Gln Glu Ala Pro Asn Gly Pro Gly Ala Glu Ala Pro Glu Glu Glu Glu
320 325 330

gaa gca gaa cct tac atg cac cga gca ggg aca ctg acc agt cta gag 1239
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335 340 345

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agt agc cat tca gtg ccc agc atg tcc atc agc gcc tcc agc caa agc      1287
Ser Ser His Ser Val Pro Ser Met Ser Ile Ser Ala Ser Ser Gln Ser
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agc tca gtc aac agc cta gca gat gcc tca gat aat gaa gaa gag gag      1335
Ser Ser Val Asn Ser Leu Ala Asp Ala Ser Asp Asn Glu Glu Glu Glu
                370                 375                 380

gag gag gaa gag gaa gaa gaa gag gag gag gaa gaa gaa ggc cct gaa      1383
Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Gly Pro Glu
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tcc cga gag atg gcc atg atg cag gag ggg gag cat aca gtc act tcc      1431
Ser Arg Glu Met Ala Met Met Gln Glu Gly Glu His Thr Val Thr Ser
        400                 405                 410

cac agc tcc atc atc cac cgg ctg ccg ggc tca gac aac cta tat gat      1479
His Ser Ser Ile Ile His Arg Leu Pro Gly Ser Asp Asn Leu Tyr Asp
    415                 420                 425

gat ccc tac cag cca gag atg acc cca ggt cca ctc caa cca cct gca      1527
Asp Pro Tyr Gln Pro Glu Met Thr Pro Gly Pro Leu Gln Pro Pro Ala
430                 435                 440                 445

gcc cct ccc acc tcc acc tcc tcc tct tct gct cgc cgc aga gct tat      1575
Ala Pro Pro Thr Ser Thr Ser Ser Ser Ser Ala Arg Arg Arg Ala Tyr
                450                 455                 460

tgc cgc aac cga gac cac ttt gcc acc atc cgt act gcc tcc ctg gtc      1623
Cys Arg Asn Arg Asp His Phe Ala Thr Ile Arg Thr Ala Ser Leu Val
            465                 470                 475

agc cgt cag atc cag gag cat gag cag gac tcg gcc ctg cgg gag caa      1671
Ser Arg Gln Ile Gln Glu His Glu Gln Asp Ser Ala Leu Arg Glu Gln
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cta agt ggc tac aag cgg atg cgg cgt cag cac cag aag caa ctg ctg      1719
Leu Ser Gly Tyr Lys Arg Met Arg Arg Gln His Gln Lys Gln Leu Leu
    495                 500                 505

gcc ctg gag tcc cgt ctg agg ggt gaa cgt gag gag cac agt ggg cgg      1767
Ala Leu Glu Ser Arg Leu Arg Gly Glu Arg Glu Glu His Ser Gly Arg
510                 515                 520                 525

ttg cag cgt gaa ctc gag gca cag cgg gct ggc ttt ggg act gag gct      1815
Leu Gln Arg Glu Leu Glu Ala Gln Arg Ala Gly Phe Gly Thr Glu Ala
                530                 535                 540

gag aag ctg gcc cgg agg cac cag gcc att ggt gag aag gaa gca cga      1863
Glu Lys Leu Ala Arg Arg His Gln Ala Ile Gly Glu Lys Glu Ala Arg
            545                 550                 555

gct gct cag gct gag gag cgg aag ttc cag cag cac atc ttg ggg cag      1911
Ala Ala Gln Ala Glu Glu Arg Lys Phe Gln Gln His Ile Leu Gly Gln
        560                 565                 570

cag aag aag gaa ctg gct gcc ctg ctg gag gca cag aag cga acc tat      1959
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    575                 580                 585

aag ctt cgg aag gag cag ttg aaa gag gag ctc cag gag aac cct agc      2007
Lys Leu Arg Lys Glu Gln Leu Lys Glu Glu Leu Gln Glu Asn Pro Ser
590                 595                 600                 605

aca ccc aaa cga gag aag gct gag tgg ctg ttg agg cag aaa gag cag      2055
Thr Pro Lys Arg Glu Lys Ala Glu Trp Leu Leu Arg Gln Lys Glu Gln
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ttg caa cag tgc cag gca gag gag gag gca ggg cta ctg cgg agg caa      2103
Leu Gln Gln Cys Gln Ala Glu Glu Glu Ala Gly Leu Leu Arg Arg Gln
            625                 630                 635

cgc cag tac ttt gag ctt cag tgt cgc caa tac aag cgc aag atg cta      2151
Arg Gln Tyr Phe Glu Leu Gln Cys Arg Gln Tyr Lys Arg Lys Met Leu
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ctg gct cgg cac agc cta gac cag gac ctg ctt cga gag gac ttg aat      2199
Leu Ala Arg His Ser Leu Asp Gln Asp Leu Leu Arg Glu Asp Leu Asn
    655                 660                 665

aag aaa cag aca cag aag gac ttg gag tgt gct ctg ctg tta cgg cag      2247
Lys Lys Gln Thr Gln Lys Asp Leu Glu Cys Ala Leu Leu Leu Arg Gln
670                 675                 680                 685

cat gag gct acc cga gag ctg gag cta cga cag ctc cag gct gtc cag      2295
His Glu Ala Thr Arg Glu Leu Glu Leu Arg Gln Leu Gln Ala Val Gln
                690                 695                 700

cgc aca cgt gct gaa ctc acc cgc ctt cag cac cag aca gag cta ggc      2343
Arg Thr Arg Ala Glu Leu Thr Arg Leu Gln His Gln Thr Glu Leu Gly
            705                 710                 715

aac cag ttg gag tac aac aag cga cgg gag caa gag ttg cgg cag aag      2391
Asn Gln Leu Glu Tyr Asn Lys Arg Arg Glu Gln Glu Leu Arg Gln Lys
        720                 725                 730

cac gcg gcc cag gtt cgc cag cag ccc aag agc ctc aaa gta cgt gca      2439
His Ala Ala Gln Val Arg Gln Gln Pro Lys Ser Leu Lys Val Arg Ala
    735                 740                 745

ggc cag cta ccc atg ggc ctc cct gct acc ggg gct ctg gga cca ctc      2487
Gly Gln Leu Pro Met Gly Leu Pro Ala Thr Gly Ala Leu Gly Pro Leu
750                 755                 760                 765

agc aca ggc acc ctt agt gaa gag cag ccc tgc tca tct ggc cag gag      2535
Ser Thr Gly Thr Leu Ser Glu Glu Gln Pro Cys Ser Ser Gly Gln Glu
                770                 775                 780

gca atc ctg ggc caa agg atg ctg gga gag gag gag gaa gca gtg cca      2583
Ala Ile Leu Gly Gln Arg Met Leu Gly Glu Glu Glu Glu Ala Val Pro
            785                 790                 795

gag aga atg att ctg gga aag gaa ggg act act ttg gag cca gag gag      2631
Glu Arg Met Ile Leu Gly Lys Glu Gly Thr Thr Leu Glu Pro Glu Glu
        800                 805                 810
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aaa cat agg agt ctg gtt aat gag gaa gat tgg gat ata tct aaa gaa 2727
Lys His Arg Ser Leu Val Asn Glu Glu Asp Trp Asp Ile Ser Lys Glu
830 835 840 845

atg aag gag agt aga gtc cca tcc ctg gca tcc cag gag aga aat att 2775
Met Lys Glu Ser Arg Val Pro Ser Leu Ala Ser Gln Glu Arg Asn Ile
850 855 860

att ggc cag gaa gag gct ggg gca tgg aat ctg tgg gag aag gag cat 2823
Ile Gly Gln Glu Glu Ala Gly Ala Trp Asn Leu Trp Glu Lys Glu His
865 870 875

gga aac ctt gtg gat atg gag ttc aag ctt ggc tgg gtc cag ggt cca 2871
Gly Asn Leu Val Asp Met Glu Phe Lys Leu Gly Trp Val Gln Gly Pro
880 885 890

gtt ctg act cca gtg cct gag gag gaa gag gag gag gaa gag gag gga 2919
Val Leu Thr Pro Val Pro Glu Glu Glu Glu Glu Glu Glu Glu Glu Gly
895 900 905

ggg gct cca att gga acc ccc agg gac cct gga gat ggc tgt cct tcc 2967
Gly Ala Pro Ile Gly Thr Pro Arg Asp Pro Gly Asp Gly Cys Pro Ser
910 915 920 925

cca gat atc ccc cca gag cca cct cca tca cat ctg aga cag tac cct 3015
Pro Asp Ile Pro Pro Glu Pro Pro Pro Ser His Leu Arg Gln Tyr Pro
930 935 940

gct agc cag ctt cct gga ttc ttg tct cat ggc ctc ctg act ggc ctc 3063
Ala Ser Gln Leu Pro Gly Phe Leu Ser His Gly Leu Leu Thr Gly Leu
945 950 955

tcc ttt gca gtg ggg tcc tcc tct ggc ctc ttg ccc cta cta ctt ctg 3111
Ser Phe Ala Val Gly Ser Ser Ser Gly Leu Leu Pro Leu Leu Leu Leu
960 965 970

ctg cta ctc cca ttg ctg gca gcc cag ggt gga ggt ggc ttg cag gca 3159
Leu Leu Leu Pro Leu Leu Ala Ala Gln Gly Gly Gly Gly Leu Gln Ala
975 980 985

gca ctg ctg gcc ctt gag gta gga cta gtg ggc ctg ggg gcc tcc tac 3207
Ala Leu Leu Ala Leu Glu Val Gly Leu Val Gly Leu Gly Ala Ser Tyr
990 995 1000 1005

ctg ttc ctt tgt aca gct cta cac ctg cca ccc agt ctg ttc tta ctc 3255
Leu Phe Leu Cys Thr Ala Leu His Leu Pro Pro Ser Leu Phe Leu Leu
1010 1015 1020

ctg gct cag ggc act gca ctg ggg gct gtc ctt agc ctg agc tgg cgc 3303
Leu Ala Gln Gly Thr Ala Leu Gly Ala Val Leu Ser Leu Ser Trp Arg
1025 1030 1035

aga ggc ctt atg ggt gtg cct ctg ggc ctt ggg gct gcc tgg ctc cta 3351
Arg Gly Leu Met Gly Val Pro Leu Gly Leu Gly Ala Ala Trp Leu Leu

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        1040                1045                1050

gct tgg ccc agc ctg gct tta cct ctg gca gct atg gcg gct ggg ggc      3399
Ala Trp Pro Ser Leu Ala Leu Pro Leu Ala Ala Met Ala Ala Gly Gly
    1055                1060                1065

aaa tgg gta cgg cag caa ggc ccc cag atg cgt cgg ggc atc tct cga      3447
Lys Trp Val Arg Gln Gln Gly Pro Gln Met Arg Arg Gly Ile Ser Arg
1070                1075                1080                1085

ctc tgg ttg cgg gtt ctg cta cgc ctg tca ccc atg gtc ttt cgg gcc      3495
Leu Trp Leu Arg Val Leu Leu Arg Leu Ser Pro Met Val Phe Arg Ala
                1090                1095                1100

cta cag ggc tgt gcg gct gtg gga gac cgg ggg ctg ttt gcc ctg tac      3543
Leu Gln Gly Cys Ala Ala Val Gly Asp Arg Gly Leu Phe Ala Leu Tyr
            1105                1110                1115

cct aag acc aat aag aat ggt ttc cga agt cga ctg cct gtc cct tgg      3591
Pro Lys Thr Asn Lys Asn Gly Phe Arg Ser Arg Leu Pro Val Pro Trp
        1120                1125                1130

ccc cgt cag gga aat cct cgc act aca cag cac cca cta gct ctg tta      3639
Pro Arg Gln Gly Asn Pro Arg Thr Thr Gln His Pro Leu Ala Leu Leu
    1135                1140                1145

gca aga gtt tgg gct ctg tgc aag ggc tgg aac tgg cgc cta gca cgg      3687
Ala Arg Val Trp Ala Leu Cys Lys Gly Trp Asn Trp Arg Leu Ala Arg
1150                1155                1160                1165

gct agc cat aga tta gct tct tgt ttg ccc ccc tgg gct gtt cat ata      3735
Ala Ser His Arg Leu Ala Ser Cys Leu Pro Pro Trp Ala Val His Ile
                1170                1175                1180

cta gct agc tgg ggc ctg ctt aag ggt gaa agg ccc agt cgg atc cct      3783
Leu Ala Ser Trp Gly Leu Leu Lys Gly Glu Arg Pro Ser Arg Ile Pro
            1185                1190                1195

cgg ctg cta ccg cga agc caa cgc cgt ctt ggg ctc tca gct tcc cga      3831
Arg Leu Leu Pro Arg Ser Gln Arg Arg Leu Gly Leu Ser Ala Ser Arg
        1200                1205                1210

cag cta cca cca ggg act gta gct ggg cgg aga tct cag acc cgc agg      3879
Gln Leu Pro Pro Gly Thr Val Ala Gly Arg Arg Ser Gln Thr Arg Arg
    1215                1220                1225

gcc ctg cct ccc tgg agg taa ccagttctaa ccctccaccc aaatttaggg         3930
Ala Leu Pro Pro Trp Arg  *
1230                1235

cattgagcac tttatctccc atgactcagt aaagtctctc cagtcccttg gcctctcctc   3990
cccttctgac ctttcttcct cagtatgttt ccccaggtcc aatcccagcc ccagatgtag   4050
atttctagac aggcagcctc ctctactgtg gagtccagaa tgacactctt gtgttttccc   4110
cagtccccta agttattgct gtcccctgct gtgtgtgtgc tcatcctcac cctcatcggc   4170
tcaggcctgg ggccaggggt ggcagggagg gaagtcatgg gggttttccc tctttgattt   4230
tgtttttctg tctcccttcc aacctgtccc cttcccctcc accaaaagag aaaaaaaaaa   4290
aaaaaaaa                                                           4298
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<210> 4
<211> 1235
<212> PRT
<213> Rattus norvegicus

<400> 4
Met Pro Ala Gly Gly Arg Ala Gly Ser Leu Lys Asp Pro Asp Val Ala
1   5   10   15
Glu Leu Phe Phe Lys Asp Asp Pro Glu Lys Leu Phe Ser Asp Leu Arg
            20   25   30
Glu Ile Gly His Gly Ser Phe Gly Ala Val Tyr Phe Ala Arg Asp Val
        35   40   45
Arg Asn Ser Glu Val Val Ala Ile Lys Lys Met Ser Tyr Ser Gly Lys
    50   55   60
Gln Ser Asn Glu Lys Trp Gln Asp Ile Ile Lys Glu Val Arg Phe Leu
65   70   75   80
Gln Lys Leu Arg His Pro Asn Thr Ile Gln Tyr Arg Gly Cys Tyr Leu
                85   90   95
Arg Glu His Thr Ala Trp Leu Val Met Glu Tyr Cys Leu Gly Ser Ala
            100   105   110
Ser Asp Leu Leu Glu Val His Lys Lys Pro Leu Gln Glu Val Glu Ile
        115   120   125
Ala Ala Val Thr His Gly Ala Leu Gln Gly Leu Ala Tyr Leu His Ser
    130   135   140
His Asn Met Ile His Arg Asp Val Lys Ala Gly Asn Ile Leu Leu Ser
145   150   155   160
Glu Pro Gly Leu Val Lys Leu Gly Asp Phe Gly Ser Ala Ser Ile Met
                165   170   175
Ala Pro Ala Asn Ser Phe Val Gly Thr Pro Tyr Trp Met Ala Pro Glu
            180   185   190
Val Ile Leu Ala Met Asp Glu Gly Gln Tyr Asp Gly Lys Val Asp Val
        195   200   205
Trp Ser Leu Gly Ile Thr Cys Ile Glu Leu Ala Glu Arg Lys Pro Pro
    210   215   220
Leu Phe Asn Met Asn Ala Met Ser Ala Leu Tyr His Ile Ala Gln Asn
225   230   235   240
Glu Ser Pro Ala Leu Gln Ser Gly His Trp Ser Glu Tyr Phe Arg Asn
                245   250   255
Phe Val Asp Ser Cys Leu Gln Lys Ile Pro Gln Asp Arg Pro Thr Ser
            260   265   270
Glu Val Leu Leu Lys His Arg Phe Val Leu Arg Glu Arg Pro Pro Thr
        275   280   285
Val Ile Met Asp Leu Ile Gln Arg Thr Lys Asp Ala Val Arg Glu Leu
    290   295   300
Asp Asn Leu Gln Tyr Arg Lys Met Lys Lys Ile Leu Phe Gln Glu Ala
305   310   315   320
Pro Asn Gly Pro Gly Ala Glu Ala Pro Glu Glu Glu Glu Glu Ala Glu
                325   330   335
Pro Tyr Met His Arg Ala Gly Thr Leu Thr Ser Leu Glu Ser Ser His
            340   345   350
Ser Val Pro Ser Met Ser Ile Ser Ala Ser Ser Gln Ser Ser Ser Val
        355   360   365
Asn Ser Leu Ala Asp Ala Ser Asp Asn Glu Glu Glu Glu Glu Glu Glu
    370   375   380
Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Gly Pro Glu Ser Arg Glu
385   390   395   400
Met Ala Met Met Gln Glu Gly Glu His Thr Val Thr Ser His Ser Ser
                405   410   415

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Ile Ile His Arg Leu Pro Gly Ser Asp Asn Leu Tyr Asp Asp Pro Tyr
            420                 425                 430
Gln Pro Glu Met Thr Pro Gly Pro Leu Gln Pro Pro Ala Ala Pro Pro
        435                 440                 445
Thr Ser Thr Ser Ser Ser Ser Ala Arg Arg Arg Ala Tyr Cys Arg Asn
    450                 455                 460
Arg Asp His Phe Ala Thr Ile Arg Thr Ala Ser Leu Val Ser Arg Gln
465                 470                 475                 480
Ile Gln Glu His Glu Gln Asp Ser Ala Leu Arg Glu Gln Leu Ser Gly
                485                 490                 495
Tyr Lys Arg Met Arg Arg Gln His Gln Lys Gln Leu Leu Ala Leu Glu
            500                 505                 510
Ser Arg Leu Arg Gly Glu Arg Glu Glu His Ser Gly Arg Leu Gln Arg
        515                 520                 525
Glu Leu Glu Ala Gln Arg Ala Gly Phe Gly Thr Glu Ala Glu Lys Leu
    530                 535                 540
Ala Arg Arg His Gln Ala Ile Gly Glu Lys Glu Ala Arg Ala Ala Gln
545                 550                 555                 560
Ala Glu Glu Arg Lys Phe Gln Gln His Ile Leu Gly Gln Gln Lys Lys
                565                 570                 575
Glu Leu Ala Ala Leu Leu Glu Ala Gln Lys Arg Thr Tyr Lys Leu Arg
            580                 585                 590
Lys Glu Gln Leu Lys Glu Glu Leu Gln Glu Asn Pro Ser Thr Pro Lys
        595                 600                 605
Arg Glu Lys Ala Glu Trp Leu Leu Arg Gln Lys Glu Gln Leu Gln Gln
    610                 615                 620
Cys Gln Ala Glu Glu Glu Ala Gly Leu Leu Arg Arg Gln Arg Gln Tyr
625                 630                 635                 640
Phe Glu Leu Gln Cys Arg Gln Tyr Lys Arg Lys Met Leu Leu Ala Arg
                645                 650                 655
His Ser Leu Asp Gln Asp Leu Leu Arg Glu Asp Leu Asn Lys Lys Gln
            660                 665                 670
Thr Gln Lys Asp Leu Glu Cys Ala Leu Leu Leu Arg Gln His Glu Ala
        675                 680                 685
Thr Arg Glu Leu Glu Leu Arg Gln Leu Gln Ala Val Gln Arg Thr Arg
    690                 695                 700
Ala Glu Leu Thr Arg Leu Gln His Gln Thr Glu Leu Gly Asn Gln Leu
705                 710                 715                 720
Glu Tyr Asn Lys Arg Arg Glu Gln Glu Leu Arg Gln Lys His Ala Ala
                725                 730                 735
Gln Val Arg Gln Gln Pro Lys Ser Leu Lys Val Arg Ala Gly Gln Leu
            740                 745                 750
Pro Met Gly Leu Pro Ala Thr Gly Ala Leu Gly Pro Leu Ser Thr Gly
        755                 760                 765
Thr Leu Ser Glu Glu Gln Pro Cys Ser Ser Gly Gln Glu Ala Ile Leu
    770                 775                 780
Gly Gln Arg Met Leu Gly Glu Glu Glu Glu Ala Val Pro Glu Arg Met
785                 790                 795                 800
Ile Leu Gly Lys Glu Gly Thr Thr Leu Glu Pro Glu Glu Gln Arg Ile
                805                 810                 815
Leu Gly Glu Glu Met Gly Thr Phe Ser Ser Ser Pro Gln Lys His Arg
            820                 825                 830
Ser Leu Val Asn Glu Glu Asp Trp Asp Ile Ser Lys Glu Met Lys Glu
        835                 840                 845
Ser Arg Val Pro Ser Leu Ala Ser Gln Glu Arg Asn Ile Ile Gly Gln
    850                 855                 860
Glu Glu Ala Gly Ala Trp Asn Leu Trp Glu Lys Glu His Gly Asn Leu
865                 870                 875                 880
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Val Asp Met Glu Phe Lys Leu Gly Trp Val Gln Gly Pro Val Leu Thr
885 890 895

Pro Val Pro Glu Glu Glu Glu Glu Glu Glu Glu Glu Gly Gly Ala Pro
900 905 910

Ile Gly Thr Pro Arg Asp Pro Gly Asp Gly Cys Pro Ser Pro Asp Ile
915 920 925

Pro Pro Glu Pro Pro Pro Ser His Leu Arg Gln Tyr Pro Ala Ser Gln
930 935 940

Leu Pro Gly Phe Leu Ser His Gly Leu Leu Thr Gly Leu Ser Phe Ala
945 950 955 960

Val Gly Ser Ser Ser Gly Leu Leu Pro Leu Leu Leu Leu Leu Leu Leu
965 970 975

Pro Leu Leu Ala Ala Gln Gly Gly Gly Gly Leu Gln Ala Ala Leu Leu
980 985 990

Ala Leu Glu Val Gly Leu Val Gly Leu Gly Ala Ser Tyr Leu Phe Leu
995 1000 1005

Cys Thr Ala Leu His Leu Pro Pro Ser Leu Phe Leu Leu Leu Ala Gln
1010 1015 1020

Gly Thr Ala Leu Gly Ala Val Leu Ser Leu Ser Trp Arg Arg Gly Leu
1025 1030 1035 1040

Met Gly Val Pro Leu Gly Leu Gly Ala Ala Trp Leu Leu Ala Trp Pro
1045 1050 1055

Ser Leu Ala Leu Pro Leu Ala Ala Met Ala Ala Gly Gly Lys Trp Val
1060 1065 1070

Arg Gln Gln Gly Pro Gln Met Arg Arg Gly Ile Ser Arg Leu Trp Leu
1075 1080 1085

Arg Val Leu Leu Arg Leu Ser Pro Met Val Phe Arg Ala Leu Gln Gly
1090 1095 1100

Cys Ala Ala Val Gly Asp Arg Gly Leu Phe Ala Leu Tyr Pro Lys Thr
1105 1110 1115 1120

Asn Lys Asn Gly Phe Arg Ser Arg Leu Pro Val Pro Trp Pro Arg Gln
1125 1130 1135

Gly Asn Pro Arg Thr Thr Gln His Pro Leu Ala Leu Leu Ala Arg Val
1140 1145 1150

Trp Ala Leu Cys Lys Gly Trp Asn Trp Arg Leu Ala Arg Ala Ser His
1155 1160 1165

Arg Leu Ala Ser Cys Leu Pro Pro Trp Ala Val His Ile Leu Ala Ser
1170 1175 1180

Trp Gly Leu Leu Lys Gly Glu Arg Pro Ser Arg Ile Pro Arg Leu Leu
1185 1190 1195 1200

Pro Arg Ser Gln Arg Arg Leu Gly Leu Ser Ala Ser Arg Gln Leu Pro
1205 1210 1215

Pro Gly Thr Val Ala Gly Arg Arg Ser Gln Thr Arg Arg Ala Leu Pro
1220 1225 1230

Pro Trp Arg
1235

<210> 5
<211> 414
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(414)
<223> N = a, c, t, g

<400> 5

acgantcacc agttggaagt tactccaaag aatgagcaca aaacaatctt aaagacactg 60
aaagatgagc agacaagaaa acttgccatt tnggcagagc agtatgaaca gagtataaat 120
gaaatgatgg cctctcangc gttacggcta gatgaggctc aagaagcaga atgccaggcc 180
ttgaggctac agctccagca ggaaatggag ctgctcaacg cctaccagag caaaatcaag 240
atgcaaacag aggcacaaca tgaacgtgag ctccagaagc tagagcagag agtgtctctg 300
cgcagagcac accttgagca gaagattgaa gaggagctgg ctgcccttca gaaggaacgc 360
agcgagagaa taaagaacct attggaaagg caagagcgag agattggaaa cttt 414

<210> 6
<211> 314
<212> DNA
<213> Homo sapien

<400> 6
gaacaaagtc atgccttaat agttctgctg atgttggcct ttcctgaggt attttctgca 60
agcagtaatc aacaaatctc ctaaaggagt ctgtccattc attagactgt aacgttgggg 120
agtcattctg ggcaatgtga tataaggcac tcattgcatt catgttgaaa aggggcggct 180
tccgttccgc caattcaata caagtgatgc caagtgacca aatatcaact ttcccatcat 240
actgtccttc atccatagct aagatcacct ctggagccat ccagtaaggt gtgcccacga 300
aggagttggc cagg 314

<210> 7
<211> 370
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(370)
<223> N = a, c, t, g

<400> 7
accaaattcc caaatcccat tctgaggctc tccatgtcaa aagtttcaat ctctcgctct 60
tgcctttcca ataggttctt tattctctcg ctgcgttcct tctgaagggc agccagctcc 120
tcttcaatct tctgctcaag gtgtggtctg cgcagagaca ctctctgctc tagcttctgg 180
agctcacgtt catgttgtgc ctctgttngn atcttgattt ggntctggta ggcgttgagc 240
agctccattt cctgctggag ctgtagcctc aaggcctggc attctgcttc ttgagcctca 300
tctagccgta acgcttgaga ggccatcatt tcatttatac tctgttcata ctgctctgcc 360
aaaatggcaa 370

<210> 8
<211> 190
<212> DNA
<213> Homo sapien

<400> 8
caacagcaga aaaacttaaa ggccatggaa atgcaaatta aaaaacagtt tcaggacact 60
tgcaaagtac agaccaaaca gtataaagca ctcaagaatc accagttgga agttactcca 120
aagaatgagc acaaaacaat cttaaagaca ctgaaagatg agcagacaag aaaacttgcc 180
attttggcag 190

<210> 9
<211> 65
<212> DNA
<213> Homo sapien

<400> 9
gagcagtatg aacagagtat aaatgaaatg atggcctctc aagcgttacg gctagatgag 60

gctca 65

<210> 10
<211> 219
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(219)
<223> N = a, c, t, g

<400> 10
acgagtcccc ccgagagcta gagtacaggc agctgcacac gttacagaag ctacgcatgg 60
atctgatccg tttacagcac cagacggaac tggaaaacca gctggagtac aataagaggc 120
gagaaagaga actgcacaga aagcatgtca tggaacttcg gcaacagcca aaaaacttaa 180
aggccatgga antgcaattt aaaaaacagt tccaggaaa 219

<210> 11
<211> 85
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(85)
<223> N = a, c, t, g

<400> 11
gtgcatatgg tatatttnat tcatttttgt aaagcgttct gttttgtgtt tactaattgg 60
gatgtcatag tacttggctg ccggg 85

<210> 12
<211> 46
<212> DNA
<213> Homo sapien

<400> 12
ctcacttggg tactacagtg tggaagctga gtgcatatgg tatatt 46

<210> 13
<211> 116
<212> DNA
<213> Homo sapien

<400> 13
gatatttggt cattgggtat cacgtgtata gagctggccg aacgtcgtcc accattgttc 60
agtatgaatg caatgtctgc cctctaccat attgctcaaa atgatcctcc aactct 116

<210> 14
<211> 118
<212> DNA
<213> Homo sapien

<400> 14
ctgaaaggcc tggattatct gcactcagag cgcaagatcc accgagatat caaagctgcc 60
aacgtgctgc tctcggagca gggtgatgtg aagatggcag acttcggtgt ggctggca 118

<210> 15
<211> 110
<212> DNA
<213> Homo sapien

<400> 15
gacccagagg aactcttcac caagcttgac cgcattggca aaggctcatt tggggaggtg 60
tacaagggga tcgacaacca caccaaggaa gtggtggcca tcaagatcat 110

<210> 16
<211> 134
<212> DNA
<213> Homo sapien

<400> 16
tcaggattct ggagctctgg agttccatta gtggctatca gatacaatgc cctgagtgga 60
ttttcattaa ggtaaggggg ttcaccttcc accatttcaa ttgccataat tccaagagac 120
cagatatcaa cttt 134

<210> 17
<211> 278
<212> PRT
<213> Saccharomyces cerevisiae

<400> 17
Met Ala Pro Ala Val Leu Gln Lys Pro Gly Val Ile Lys Asp Pro Ser
1 5 10 15
Ile Ala Ala Leu Phe Ser Asn Lys Asp Pro Glu Gln Asp Leu Arg Glu
20 25 30
Ile Gly His Gly Ser Phe Gly Ala Val Tyr Phe Ala Tyr Asp Lys Lys
35 40 45
Asn Glu Gln Thr Val Ala Ile Lys Lys Met Asn Phe Ser Gly Lys Gln
50 55 60
Ala Val Glu Lys Trp Asn Asp Ile Leu Lys Glu Val Ser Phe Leu Asn
65 70 75 80
Thr Val Val His Pro His Ile Val Asp Tyr Lys Ala Cys Phe Leu Lys
85 90 95
Asp Thr Thr Cys Trp Leu Val Met Glu Tyr Cys Ile Gly Ser Ala Ala
100 105 110
Asp Ile Val Asp Val Leu Arg Lys Gly Met Arg Glu Val Glu Ile Ala
115 120 125
Ala Ile Cys Ser Gln Thr Leu Asp Ala Leu Arg Tyr Leu His Ser Leu
130 135 140
Lys Arg Ile His Arg Asp Ile Lys Ala Gly Asn Ile Leu Leu Ser Asp
145 150 155 160
His Ala Ile Val Lys Leu Ala Asp Phe Gly Ser Ala Ser Leu Val Asp
165 170 175
Pro Ala Gln Thr Phe Ile Gly Thr Pro Phe Phe Met Ala Pro Glu Val
180 185 190
Ile Leu Ala Met Asp Glu Gly His Tyr Thr Asp Arg Ala Asp Ile Trp
195 200 205
Ser Leu Gly Ile Thr Cys Ile Glu Leu Ala Glu Arg Arg Pro Pro Leu
210 215 220
Phe Ser Met Asn Ala Met Ser Ala Leu Tyr His Ile Ala Gln Asn Asp
225 230 235 240
Pro Pro Thr Leu Ser Pro Ile Asp Thr Ser Glu Gln Pro Glu Trp Ser
245 250 255
Leu Glu Phe Val Gln Phe Ile Asp Lys Cys Leu Arg Lys Pro Ala Glu

260 265 270
Glu Arg Met Ser Ala Glu
275

<210> 18
<211> 273
<212> PRT
<213> C. elegans

<400> 18
Arg Glu Glu Arg Glu Arg Arg Lys Lys Gln Leu Tyr Ala Lys Leu Asn
1 5 10 15
Glu Ile Cys Ser Asp Gly Asp Pro Ser Thr Lys Tyr Ala Asn Leu Val
20 25 30
Lys Ile Gly Gln Gly Ala Ser Gly Gly Val Tyr Thr Ala Tyr Glu Ile
35 40 45
Gly Thr Asn Val Ser Val Ala Ile Lys Gln Met Asn Leu Glu Lys Gln
50 55 60
Pro Lys Lys Glu Leu Ile Ile Asn Glu Ile Leu Val Met Lys Gly Ser
65 70 75 80
Lys His Pro Asn Ile Val Asn Phe Ile Asp Ser Tyr Val Leu Lys Gly
85 90 95
Asp Leu Trp Val Ile Met Glu Tyr Met Glu Gly Gly Ser Leu Thr Val
100 105 110
Asp Val Val Thr His Cys Ile Leu Thr Glu Gly Gln Ile Gly Ala Val
115 120 125
Cys Arg Glu Thr Leu Ser Gly Leu Glu Phe Leu His Ser Lys Gly Val
130 135 140
Leu His Arg Asp Ile Lys Ser Asp Asn Ile Leu Leu Ser Met Glu Gly
145 150 155 160
Asp Ile Lys Leu Thr Asp Phe Gly Phe Cys Ala Gln Ile Asn Glu Leu
165 170 175
Asn Leu Lys Arg Thr Thr Met Val Gly Thr Pro Tyr Trp Met Ala Pro
180 185 190
Glu Val Val Ser Arg Lys Glu Tyr Gly Pro Lys Val Asp Ile Trp Ser
195 200 205
Leu Gly Ile Met Ile Ile Glu Met Ile Glu Gly Glu Pro Pro Tyr Leu
210 215 220
Asn Glu Thr Pro Leu Arg Ala Leu Tyr Leu Ile Ala Thr Asn Gly Thr
225 230 235 240
Pro Lys Leu Lys Glu Pro Glu Asn Leu Ser Ser Ser Leu Lys Lys Phe
245 250 255
Leu Asp Trp Cys Leu Cys Cys Val Glu Pro Glu Asp Arg Ala Ser Ala
260 265 270
Thr

<210> 19
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (1)...(33)
<223> N = inosineI

<223> Oligonucleotide primers

<400> 19
gacgctggat ccaaagatac tggncaaggg ngc 33

<210> 20
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (1)...(21)
<223> N = inosineI

<223> Oligonucleotide primers

<400> 20
ggngtnccag ttngtngcna t 21

<210> 21
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (1)...(28)
<223> N = inosineI

<223> Oligonucleotide primers

<400> 21
aaaggaagca nagncagnaa cggaagat 28

<210> 22
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (1)...(30)
<223> N = iinosineI

<223> Oligonucleotide primers

<400> 22
gacgctgaat tcaccttcng gngccatcca 30

<210> 23
<211> 20
<212> PRT
<213> Rattus norvegicus

<400> 23
Thr Lys Asp Ala Val Arg Glu Leu Asp Asn Leu Gln Tyr Arg Lys Met
 1   5   10   15
Lys Lys Leu Leu

20

<210> 24
<211> 19
<212> PRT
<213> Rattus norvegicus

<400> 24
Lys Lys Glu Leu Asn Ser Phe Leu Glu Ser Gln Lys Arg Glu Tyr Lys
1 5 10 15
Leu Arg Lys

<210> 25
<211> 20
<212> PRT
<213> Rattus norvegicus

<400> 25
Arg Glu Leu Arg Glu Leu Glu Gln Arg Val Ser Leu Arg Arg Ala Leu
1 5 10 15
Leu Glu Gln Lys
20

<210> 26
<211> 8
<212> PRT
<213> Rattus norvegicus

<400> 26
His Arg Asp Ile Lys Ala Gly Asn
1 5

<210> 27
<211> 3781
<212> DNA
<213> C. elegans

<220>
<221> CDS
<222> (279)...(3227)

<400> 27
caagtttgag agtttctcac atcaaccagt tgcatccaca attttctcga tattatctgc 60
aaggaatcct ctggctaatg tgctctctcc gatcgacgac ggcgacgcca tcctcgattt 120
ttactcatcc atcaacaaca aaatcaaaca agacaagaaa aacatcagaa aatttcaaaa 180
attaataaca atacacattt attaataatc aaaaattcat tttcgttggc gccgcgcttc 240
tcgaatatac ggagaacgga ggaggtggtg gagttacg atg gcg cct gcc gtc tta 296
Met Ala Pro Ala Val Leu
1 5

caa aaa ccc ggt gtt atc aag gat cca tcg att gct gca ttg ttc agt 344
Gln Lys Pro Gly Val Ile Lys Asp Pro Ser Ile Ala Ala Leu Phe Ser
10 15 20

aat aag gat cca gag cag aga tat caa gat tta aga gaa att gga cat 392
Asn Lys Asp Pro Glu Gln Arg Tyr Gln Asp Leu Arg Glu Ile Gly His
25 30 35

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gga tct ttt gga gct gtc tat ttt gca tat gac aaa aaa aat gag cag      440
Gly Ser Phe Gly Ala Val Tyr Phe Ala Tyr Asp Lys Lys Asn Glu Gln
     40                  45                  50

act gtt gcg att aaa aag atg aat ttt agt gga aaa cag gct gtc gaa      488
Thr Val Ala Ile Lys Lys Met Asn Phe Ser Gly Lys Gln Ala Val Glu
 55                  60                  65                  70

aaa tgg aat gat att ctt aaa gaa gtg tct ttt ctg aat aca gtt gtt      536
Lys Trp Asn Asp Ile Leu Lys Glu Val Ser Phe Leu Asn Thr Val Val
                 75                  80                  85

cat cca cat att gtc gac tac aag gct tgt ttt ctt aag gac act aca      584
His Pro His Ile Val Asp Tyr Lys Ala Cys Phe Leu Lys Asp Thr Thr
             90                  95                 100

tgt tgg ctt gtg atg gag tac tgt att ggc tct gca gcc gat ata gtg      632
Cys Trp Leu Val Met Glu Tyr Cys Ile Gly Ser Ala Ala Asp Ile Val
        105                 110                 115

gat gtc ttg cga aaa gga atg cga gaa gtc gaa atc gct gcg att tgc      680
Asp Val Leu Arg Lys Gly Met Arg Glu Val Glu Ile Ala Ala Ile Cys
    120                 125                 130

tct caa act ttg gat gct ctt cga tat ctt cac tct ctg aag cga ata      728
Ser Gln Thr Leu Asp Ala Leu Arg Tyr Leu His Ser Leu Lys Arg Ile
135                 140                 145                 150

cat cga gat att aaa gct gga aat att ctg cta tct gat cat gct att      776
His Arg Asp Ile Lys Ala Gly Asn Ile Leu Leu Ser Asp His Ala Ile
                155                 160                 165

gtt aaa cta gct gat ttc gga tcc gca tcc ctg gta gat ccg gct caa      824
Val Lys Leu Ala Asp Phe Gly Ser Ala Ser Leu Val Asp Pro Ala Gln
            170                 175                 180

act ttc atc gga acg ccg ttt ttc atg gcc cca gag gta att ctg gca      872
Thr Phe Ile Gly Thr Pro Phe Phe Met Ala Pro Glu Val Ile Leu Ala
        185                 190                 195

atg gat gag ggt cac tac acg gat cgt gca gat att tgg tca ttg ggt      920
Met Asp Glu Gly His Tyr Thr Asp Arg Ala Asp Ile Trp Ser Leu Gly
    200                 205                 210

atc acg tgt ata gag ctg gcc gaa cgt cgt cca cca ttg ttc agt atg      968
Ile Thr Cys Ile Glu Leu Ala Glu Arg Arg Pro Pro Leu Phe Ser Met
215                 220                 225                 230

aat gca atg tct gcc ctc tac cat att gct caa aat gat cct cca act     1016
Asn Ala Met Ser Ala Leu Tyr His Ile Ala Gln Asn Asp Pro Pro Thr
                235                 240                 245

ctt tct cca att gac act agc gaa caa ccg gaa tgg tcg ctg gaa ttc     1064
Leu Ser Pro Ile Asp Thr Ser Glu Gln Pro Glu Trp Ser Leu Glu Phe
            250                 255                 260

gtt caa ttt ata gac aaa tgt ctt cga aaa cca gca gaa gag cga atg     1112
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Val Gln Phe Ile Asp Lys Cys Leu Arg Lys Pro Ala Glu Glu Arg Met
        265                 270                 275

tca gct gaa gaa tgc ttt cga cat cca ttc att caa cgg tct cgc cca      1160
Ser Ala Glu Glu Cys Phe Arg His Pro Phe Ile Gln Arg Ser Arg Pro
    280                 285                 290

tca gac aca att cag gaa ctc att cag aga acg aaa aat atg gta tta      1208
Ser Asp Thr Ile Gln Glu Leu Ile Gln Arg Thr Lys Asn Met Val Leu
295                 300                 305                 310

gag ttg gat aat ttt caa tac aaa aag atg aga aaa ctc atg tat ttg      1256
Glu Leu Asp Asn Phe Gln Tyr Lys Lys Met Arg Lys Leu Met Tyr Leu
                315                 320                 325

gat gaa aca gaa gga aaa gaa gga agt gaa gga aat gga gca tct gat      1304
Asp Glu Thr Glu Gly Lys Glu Gly Ser Glu Gly Asn Gly Ala Ser Asp
            330                 335                 340

gat tta gat ttt cat gga aat gaa gct aat tca att gga aga gca gga      1352
Asp Leu Asp Phe His Gly Asn Glu Ala Asn Ser Ile Gly Arg Ala Gly
        345                 350                 355

gat tct gcg tca tct cga agt gct tct ctt act tct ttc cga tca atg      1400
Asp Ser Ala Ser Ser Arg Ser Ala Ser Leu Thr Ser Phe Arg Ser Met
    360                 365                 370

cag agt agt gga gga gct ggt ctt tta gtg tcc acc aat acg acg ggt      1448
Gln Ser Ser Gly Gly Ala Gly Leu Leu Val Ser Thr Asn Thr Thr Gly
375                 380                 385                 390

gct atg gat aat gtg cat gga tcc tct gga tac ggt aat gga agt agt      1496
Ala Met Asp Asn Val His Gly Ser Ser Gly Tyr Gly Asn Gly Ser Ser
                395                 400                 405

tcg acg acg agc tcc gca cgc cgc cgt cct cca att cct tcg caa atg      1544
Ser Thr Thr Ser Ser Ala Arg Arg Arg Pro Pro Ile Pro Ser Gln Met
            410                 415                 420

ctc tct tct aca tca acg tct ggt gtt gga act atg ccg agt cat gga      1592
Leu Ser Ser Thr Ser Thr Ser Gly Val Gly Thr Met Pro Ser His Gly
        425                 430                 435

tca gtt gga gca tcg att acg gcg atc gca gtc aat cca aca ccg tct      1640
Ser Val Gly Ala Ser Ile Thr Ala Ile Ala Val Asn Pro Thr Pro Ser
    440                 445                 450

cct tca gaa cct atc cca aca tca caa cca aca tcg aaa tca gaa tca      1688
Pro Ser Glu Pro Ile Pro Thr Ser Gln Pro Thr Ser Lys Ser Glu Ser
455                 460                 465                 470

tct tct ata ctc gaa act gca cac gat gat cct ttg gac acg tcg ata      1736
Ser Ser Ile Leu Glu Thr Ala His Asp Asp Pro Leu Asp Thr Ser Ile
                475                 480                 485

cgt gct cca gtg aaa gac ttg cat atg ccg cat cga gca gtc aag gaa      1784
Arg Ala Pro Val Lys Asp Leu His Met Pro His Arg Ala Val Lys Glu
            490                 495                 500
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cga ata gcc acg ttg caa aat cac aaa ttc gcg acg ctt cgt tcc cag      1832
Arg Ile Ala Thr Leu Gln Asn His Lys Phe Ala Thr Leu Arg Ser Gln
        505                 510                 515

aga ata atc aat cag gaa caa gaa gaa tat acg aaa gag aac aat atg      1880
Arg Ile Ile Asn Gln Glu Gln Glu Glu Tyr Thr Lys Glu Asn Asn Met
    520                 525                 530

tat gag caa atg agc aag tac aag cat cta cga caa gca cat cac aaa      1928
Tyr Glu Gln Met Ser Lys Tyr Lys His Leu Arg Gln Ala His His Lys
535                 540                 545                 550

gag ctc caa caa ttt gaa gaa cga tgt gca tta gat aga gag caa ctg      1976
Glu Leu Gln Gln Phe Glu Glu Arg Cys Ala Leu Asp Arg Glu Gln Leu
                555                 560                 565

cgt gtg aaa atg gat cga gaa ctc gaa caa ttg aca acg aca tac tcg      2024
Arg Val Lys Met Asp Arg Glu Leu Glu Gln Leu Thr Thr Thr Tyr Ser
            570                 575                 580

aaa gaa aag atg aga gtg agg tgt tca cag aat aat gaa cta gac aaa      2072
Lys Glu Lys Met Arg Val Arg Cys Ser Gln Asn Asn Glu Leu Asp Lys
        585                 590                 595

cgg aaa aaa gat atc gaa gat ggg gag aaa aag atg aaa aag acg aaa      2120
Arg Lys Lys Asp Ile Glu Asp Gly Glu Lys Lys Met Lys Lys Thr Lys
    600                 605                 610

aat agt caa aat cag cag cag atg aaa ctg tat tca gcg caa caa ttg      2168
Asn Ser Gln Asn Gln Gln Gln Met Lys Leu Tyr Ser Ala Gln Gln Leu
615                 620                 625                 630

aaa gaa tac aag tat aac aag gag gca cag aaa aca cga tta cga agt      2216
Lys Glu Tyr Lys Tyr Asn Lys Glu Ala Gln Lys Thr Arg Leu Arg Ser
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ctg aac atg cct cga agt act tat gag aac gca atg aaa gaa gtg aaa      2264
Leu Asn Met Pro Arg Ser Thr Tyr Glu Asn Ala Met Lys Glu Val Lys
            650                 655                 660

gcc gat ctg aat cga gtg aaa gat gca cgg gaa aat gat ttt gac gag      2312
Ala Asp Leu Asn Arg Val Lys Asp Ala Arg Glu Asn Asp Phe Asp Glu
        665                 670                 675

aag ctt cgt gca gaa ctt gaa gat gaa att gta agg tat cgc agg caa      2360
Lys Leu Arg Ala Glu Leu Glu Asp Glu Ile Val Arg Tyr Arg Arg Gln
    680                 685                 690

caa ctc agt aat ctt cat caa ttg gaa gaa caa ttg gat gat gaa gac      2408
Gln Leu Ser Asn Leu His Gln Leu Glu Glu Gln Leu Asp Asp Glu Asp
695                 700                 705                 710

gta aac gtg caa gaa cgc caa atg gac acg cgt cac gga tta ctg tca      2456
Val Asn Val Gln Glu Arg Gln Met Asp Thr Arg His Gly Leu Leu Ser
                715                 720                 725

aag cag cat gaa atg acg cgc gat ttg gaa ata cag cat ctc aac gag      2504
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Lys Gln His Glu Met Thr Arg Asp Leu Glu Ile Gln His Leu Asn Glu
                730                 735                 740

ctt cac gcg atg aaa aaa cga cat ttg gag aca caa cac gag gcg gaa      2552
Leu His Ala Met Lys Lys Arg His Leu Glu Thr Gln His Glu Ala Glu
            745                 750                 755

tcg gca agt caa aat gag tac aca cag agg caa cag gat gaa ttg aga      2600
Ser Ala Ser Gln Asn Glu Tyr Thr Gln Arg Gln Gln Asp Glu Leu Arg
        760                 765                 770

aaa aag cat gcg atg cag tca aga caa cag cca aga gat tta aag atc      2648
Lys Lys His Ala Met Gln Ser Arg Gln Gln Pro Arg Asp Leu Lys Ile
775                 780                 785                 790

caa gaa gca caa att cga aaa caa tac cga caa gtt gtg aag act cag      2696
Gln Glu Ala Gln Ile Arg Lys Gln Tyr Arg Gln Val Val Lys Thr Gln
                795                 800                 805

act cgc caa ttt aag ctc tac ctt aca caa atg gtg caa gta gtt cca      2744
Thr Arg Gln Phe Lys Leu Tyr Leu Thr Gln Met Val Gln Val Val Pro
            810                 815                 820

aaa gat gaa caa aaa gag ctc acg tct cga cta aaa cag gat caa atg      2792
Lys Asp Glu Gln Lys Glu Leu Thr Ser Arg Leu Lys Gln Asp Gln Met
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caa aaa gtc gca ctt ctt gct tca caa tac gaa agt caa atc aaa aaa      2840
Gln Lys Val Ala Leu Leu Ala Ser Gln Tyr Glu Ser Gln Ile Lys Lys
    840                 845                 850

atg gtt cag gat aag aca gtg aag ctc gag tcg tgg caa gaa gat gaa      2888
Met Val Gln Asp Lys Thr Val Lys Leu Glu Ser Trp Gln Glu Asp Glu
855                 860                 865                 870

caa cgg gtt ctt agt gag aag ttg gag aaa gaa ttg gaa gaa ttg att      2936
Gln Arg Val Leu Ser Glu Lys Leu Glu Lys Glu Leu Glu Glu Leu Ile
                875                 880                 885

gct tat cag aag aag acg aga gcc aca tta gaa gag cag att aaa aag      2984
Ala Tyr Gln Lys Lys Thr Arg Ala Thr Leu Glu Glu Gln Ile Lys Lys
            890                 895                 900

gaa cgt acg gca ctc gaa gaa cga att ggc aca cga cgt gca atg ctt      3032
Glu Arg Thr Ala Leu Glu Glu Arg Ile Gly Thr Arg Arg Ala Met Leu
        905                 910                 915

gaa cag aag att att gaa gaa cgc gaa caa atg gga gaa atg cgt cga      3080
Glu Gln Lys Ile Ile Glu Glu Arg Glu Gln Met Gly Glu Met Arg Arg
    920                 925                 930

cta aag aag gag caa atc cgt gat cga cac agt caa gaa cgc cat cgt      3128
Leu Lys Lys Glu Gln Ile Arg Asp Arg His Ser Gln Glu Arg His Arg
935                 940                 945                 950

ctc gag aat cat ttc gta cgg acg ggc tcg acg agc aga agt tct ggt      3176
Leu Glu Asn His Phe Val Arg Thr Gly Ser Thr Ser Arg Ser Ser Gly
                955                 960                 965
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Gly Ile Ala Pro Gly Val Gly Asn Ser Ser Ser Ile Gln Met Ala Met
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35 40 45
Asp Lys Lys Asn Glu Gln Thr Val Ala Ile Lys Lys Met Asn Phe Ser
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Gly Lys Gln Ala Val Glu Lys Trp Asn Asp Ile Leu Lys Glu Val Ser
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Phe Leu Asn Thr Val Val His Pro His Ile Val Asp Tyr Lys Ala Cys
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Phe Leu Lys Asp Thr Thr Cys Trp Leu Val Met Glu Tyr Cys Ile Gly
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Ser Ala Ala Asp Ile Val Asp Val Leu Arg Lys Gly Met Arg Glu Val
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Glu Ile Ala Ala Ile Cys Ser Gln Thr Leu Asp Ala Leu Arg Tyr Leu
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His Ser Leu Lys Arg Ile His Arg Asp Ile Lys Ala Gly Asn Ile Leu
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Pro Glu Val Ile Leu Ala Met Asp Glu Gly His Tyr Thr Asp Arg Ala
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Asp Ile Trp Ser Leu Gly Ile Thr Cys Ile Glu Leu Ala Glu Arg Arg
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Pro Pro Leu Phe Ser Met Asn Ala Met Ser Ala Leu Tyr His Ile Ala
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Gln Asn Asp Pro Pro Thr Leu Ser Pro Ile Asp Thr Ser Glu Gln Pro
245 250 255
Glu Trp Ser Leu Glu Phe Val Gln Phe Ile Asp Lys Cys Leu Arg Lys

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Pro Ala Glu Glu Arg Met Ser Ala Glu Glu Cys Phe Arg His Pro Phe
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Ile Gln Arg Ser Arg Pro Ser Asp Thr Ile Gln Glu Leu Ile Gln Arg
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Thr Lys Asn Met Val Leu Glu Leu Asp Asn Phe Gln Tyr Lys Lys Met
305                     310                     315                     320
Arg Lys Leu Met Tyr Leu Asp Glu Thr Glu Gly Lys Glu Gly Ser Glu
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Gly Asn Gly Ala Ser Asp Asp Leu Asp Phe His Gly Asn Glu Ala Asn
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Ser Ile Gly Arg Ala Gly Asp Ser Ala Ser Ser Arg Ser Ala Ser Leu
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Thr Ser Phe Arg Ser Met Gln Ser Ser Gly Gly Ala Gly Leu Leu Val
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Ser Thr Asn Thr Thr Gly Ala Met Asp Asn Val His Gly Ser Ser Gly
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Tyr Gly Asn Gly Ser Ser Ser Thr Thr Ser Ser Ala Arg Arg Arg Pro
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Pro Ile Pro Ser Gln Met Leu Ser Ser Thr Ser Thr Ser Gly Val Gly
                420                     425                     430
Thr Met Pro Ser His Gly Ser Val Gly Ala Ser Ile Thr Ala Ile Ala
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Val Asn Pro Thr Pro Ser Pro Ser Glu Pro Ile Pro Thr Ser Gln Pro
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Thr Ser Lys Ser Glu Ser Ser Ser Ile Leu Glu Thr Ala His Asp Asp
465                     470                     475                     480
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His Arg Ala Val Lys Glu Arg Ile Ala Thr Leu Gln Asn His Lys Phe
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Ala Thr Leu Arg Ser Gln Arg Ile Ile Asn Gln Glu Gln Glu Glu Tyr
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Arg Gln Ala His His Lys Glu Leu Gln Gln Phe Glu Glu Arg Cys Ala
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Leu Asp Arg Glu Gln Leu Arg Val Lys Met Asp Arg Glu Leu Glu Gln
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Asn Asn Glu Leu Asp Lys Arg Lys Lys Asp Ile Glu Asp Gly Glu Lys
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Lys Met Lys Lys Thr Lys Asn Ser Gln Asn Gln Gln Gln Met Lys Leu
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Tyr Ser Ala Gln Gln Leu Lys Glu Tyr Lys Tyr Asn Lys Glu Ala Gln
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Ala Met Lys Glu Val Lys Ala Asp Leu Asn Arg Val Lys Asp Ala Arg
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Val Arg Tyr Arg Arg Gln Gln Leu Ser Asn Leu His Gln Leu Glu Glu
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Gln Leu Asp Asp Glu Asp Val Asn Val Gln Glu Arg Gln Met Asp Thr
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Arg His Gly Leu Leu Ser Lys Gln His Glu Met Thr Arg Asp Leu Glu
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Gln Gln Asp Glu Leu Arg Lys Lys His Ala Met Gln Ser Arg Gln Gln
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785                 790                 795                 800
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Met Val Gln Val Val Pro Lys Asp Glu Gln Lys Glu Leu Thr Ser Arg
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Leu Lys Gln Asp Gln Met Gln Lys Val Ala Leu Leu Ala Ser Gln Tyr
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Glu Ser Gln Ile Lys Lys Met Val Gln Asp Lys Thr Val Lys Leu Glu
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Ser Trp Gln Glu Asp Glu Gln Arg Val Leu Ser Glu Lys Leu Glu Lys
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Glu Glu Gln Ile Lys Lys Glu Arg Thr Ala Leu Glu Glu Arg Ile Gly
            900                 905                 910
Thr Arg Arg Ala Met Leu Glu Gln Lys Ile Ile Glu Glu Arg Glu Gln
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Met Gly Glu Met Arg Arg Leu Lys Lys Glu Gln Ile Arg Asp Arg His
    930                 935                 940
Ser Gln Glu Arg His Arg Leu Glu Asn His Phe Val Arg Thr Gly Ser
945                 950                 955                 960
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Ser Ile Gln Met Ala Met
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